

# MICROBIC RESPIRATION

## RESPIRATION OF TRYPANOSOMA LEWISI AND LEISHMANIA TROPICA

By

MALCOLM HERMAN SOULE

A DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of  
Doctor of Science in The University of Michigan

Reprinted from

THE JOURNAL OF INFECTIOUS DISEASES, Vol. 36, No. 3, March, 1925, pp. 245-308  
CHICAGO

*An Arbor*  
1935





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## MICROBIC RESPIRATION

### III. RESPIRATION OF TRYPANOSOMA LEWISI AND LEISHMANIA TROPICA\*

*From the Hygienic Laboratory of the University of Michigan, Ann Arbor*

MALCOLM HERMAN SOULE

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Fully Developed Cultures in Air.

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#### INTRODUCTION

The pathogenic protozoa in a remarkably short time have risen from an obscure to a commanding position because of their importance in the causation of disease peculiar to the warm countries. The intermingling of troops from the whole world during the recent war gave evidence of increased infection from the known pathogenic forms. Of the many protozoa whose relation to disease have been proved, the hemoflagellates and the amebas are the most important.

In 1900, Laveran and Mesnil began their study of the trypanosomes, developing methods that led to the splendid series of researches which have come from the Pasteur Institute on these and related organisms.

Of no less importance to the protistologists was the cultivation on artificial mediums of Tr. lewisi by Novy and MacNeal<sup>1</sup> in 1903, who obtained, for the first time, strictly pure cultures of an animal parasite.

Since then a large number of flagellates have been grown on artificial mediums, the majority on either the original medium of these authors or on some modification of it. The serial cultivation, however, of such organisms as Tr. gambiense has not been attained.

Received for publication, Nov. 1, 1924.

\* A dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Science in the University of Michigan.

<sup>1</sup> Contributions to Medical Research: Dedicated to V. C. Vaughan, Ann Arbor, Wahr, 1903, p. 549.

The vast literature of protozoa, which is growing and receiving continually new additions, is published in so many periodicals, some of them very difficult to obtain, that one hesitates and only ventures into this field with considerable trepidation.

It has been the impression among most workers that knowledge of the composition and reaction of the medium was most important. Little or no attention has been given the gaseous environment, and yet it is reasonable to believe that some recognition should be given to the respiratory changes.

Spallanzani<sup>2</sup> showed that all kinds of animals, whether they had lungs or not, gave off  $\text{CO}_2$ , consumed  $\text{O}_2$  and liberated heat, thus confirming Lavoisier's<sup>3</sup> combustion theory of respiration.

By respiration in its widest sense must be understood all those processes in the organism whereby the potential energy stored up in chemical compounds of high complexity is set free to furnish the energy required by the organism for its vital activities. This object is effected by processes of oxidation; the result is the production of energy with the formation of simple chemical substances, such as water and  $\text{CO}_2$ .

For the processes of oxidation, the organism either utilizes the free molecular  $\text{O}_2$  from its air environment, or makes use of the oxygen which is present in organic combination in the nutritive material, as is the case with anaerobic organisms which live in a medium lacking free  $\text{O}_2$ .

As the pathogenic hemoflagellates usually inhabit the blood stream, it is reasonable to believe that they would require some free  $\text{O}_2$  for their development. It must be supposed that protozoa, like bacteria and other unicellular organisms, take up the required  $\text{O}_2$  from the surrounding medium, and give off  $\text{CO}_2$ .

The effect of various atmospheres has been tried on some of the lower forms of animal organisms. The experiments of Pütter<sup>4</sup> on a number of ciliates, both free-living and parasitic, showed that when these animals were placed in an anaerobic environment different species reacted very differently to the conditions. Excess of  $\text{O}_2$  was found by this author to have an injurious effect on *Spirostomum*. Jacobs<sup>5</sup>, placing drops of water containing *Paramecium* in an Engelmann gas chamber, observed  $\text{CO}_2$  to be toxic for 12 varieties.

<sup>2</sup> Dissertazioni Varie, Vol. 2: Mémoir sulla Respirazione, 1826.

<sup>3</sup> Histoire et Mémoires de l'Acad. d. Sc., Paris (1780); Mémoires, Année, 1777, p. 185.

<sup>4</sup> Ztschr. f. allg. Physiol., 1904, 3, p. 363.

<sup>5</sup> Jour. Exp. Zool., 1912, 12, pp. 519-542.



A quantitative study of the gas exchange by protozoa has been so beset with difficulties that few investigators have ventured into the field. It has been considered sufficient to detect respiration in a qualitative way, and for this two methods have been used. The first consists in passing measured quantities of air through the infusion with subsequent absorption by  $\text{Ba}(\text{OH})_2$  of the  $\text{CO}_2$  which is formed; the second, in observing changes in the color of indicator solutions which contain the organisms. The first method is truly qualitative in that no effort is made to measure the respiration of one particular species, and the second is obviously limited to the use of clear liquids on which many organisms do not grow. It is therefore readily understandable why no accurate measurements have been made.

It follows that quantitative information concerning the gaseous requirements of the protozoa is of importance. In the work which follows accurate estimations have been made, for the first time, of the respiratory changes induced by pure cultures of 2 species of pathogenic flagellates. Their growth has been measured by manometric observations, and the gas changes have been determined by exact quantitative analysis. For these organisms, the problem of the gas exchange has been satisfactorily solved. This work is one of a series of studies on microbial respiration which has been carried on in this laboratory for several years. The respiration of pure cultures of other protozoa will be taken up in subsequent papers.

*Tr. lewisi*, although not a strictly pathogenic form as found in the common rat, is an excellent type of the whole group. Owing to the almost universal distribution of its host, it has been found in all parts of the world. Since no difficulty is experienced in carrying the organism through a large series of subcultures, it lent itself very well to our study. The strain used in this work was isolated from an infected rat, and at present the culture is in its one hundred and twentieth generation.

*L. tropica* was first described by Cunningham<sup>6</sup> (1885). In view of the fact that his description was imperfect, most authors give the credit of discovering the parasite to Wright<sup>7</sup> (1903), who demonstrated it to be the cause of oriental sore. It is one of the strictly pathogenic protozoa which can be successfully cultivated and with

<sup>6</sup> Hegner and Taliaferro: Human Protozoology, New York, Macmillan, 1924, p. 193.

<sup>7</sup> Jour. Med. Res., 1903, 10, p. 472.

ease. The strain used in this work was obtained from Dr. W. H. Brown of the Rockefeller Institute, and is now in the two hundred and thirteenth generation in this laboratory. We believe that the information concerning the gas requirements or respiratory changes of these two organisms will be of much value in the further study of other pathogenic forms.

#### METHODS

*Culture Medium.*—Finely comminuted choice lean beef was added to 2 parts of distilled water. The meat was extracted for 24 hours in the icebox, then strained, after which the liquid was heated to coagulate the protein and filtered.

To the meat extract, thus prepared, 1% of Witte's peptone and 0.5% of sodium chloride (Kahlbaum) were added. The liquid was then carefully adjusted to  $P_H$  7.4 with NaOH; boiled, filtered, and 2% of agar added.

The agar medium was then filled into a number of 125 c.c. sterile Erlenmeyer flasks, and autoclaved at 110 C. for 20 minutes. For a given experiment, 1 or 2 of the flasks were liquefied by heat. As is known, the  $P_H$  of a medium changes on standing, and, since the stock medium was kept for some weeks, the  $P_H$  was again carefully adjusted just before being tubed. The medium was measured, by means of a standard pipet, into sterilized tubes, which were then autoclaved at 110 C. for 20 minutes; after this the tubes were cooled to about 50 C., and an equal volume of defibrinated rabbit blood or serum was added; the whole was then well mixed and slanted.

The blood was obtained by bleeding rabbits from the carotid using the technic of Novy;<sup>8</sup> after defibrination, the blood was placed in the icebox for at least 2 days to avoid the germicidal effects, to which attention was called by Behrens.<sup>9</sup>

In order to insure sufficient inoculation, the transplantation was always made by means of a capillary pipet; 1 drop of the water of condensation being usually transferred and then spread over the surface of the medium with a sterile platinum wire to insure as even an inoculation as possible. Obviously, for purposes of comparison, it is desirable to inoculate approximately the same number of viable organisms. This, however, is seldom, if ever, realized, and the irregularities in manometric readings which may be noted in parallel tests find an explanation in the varying number of organisms at work, though the volume of air present is also a factor.

*Culture Tubes.*—An important consideration in the matter of the culture tube is the volume of the contained air. While it is possible to obtain manometric readings with small tubes, they are not suitable for the withdrawal of 10-20 c.c. of the gas for analysis. Hence, it was necessary to use the larger tubes described in Part I.

The plain tubes (20 x 200 mm.) of resistance glass were, at times, employed. When empty, the air capacity of such tubes ranged from 55 to 60 c.c. Shorter tubes, 20 x 150 mm., were necessary whenever the anaerobe jar was used.

The *h*-tube (fig. 1B, Part I) with an air volume of about 100 c.c. was usually used. The side tube permitted the introduction of alkali for absorption of CO<sub>2</sub>, or of H<sub>2</sub>O, or of any other desired reagent.

<sup>8</sup> Laboratory Work in Bacteriology, Ann Arbor, Wahr, 1899, p. 460. Jour. Infect. Dis., 1917, 20, p. 502.

<sup>9</sup> Jour. Infect. Dis., 1914, 15, pp. 24-62.



Side-arm tubes (fig. 2C, Part I) were found to be very useful, especially in experiments in which an all-glass seal was desired.

The inoculated *h*-tubes were attached to the slanted tip of manometers by means of selected rubber stoppers treated with glycerol, of No. 3 or 4 size. A dry stopper does not give a perfect contact with the glass, and as a result leakage occurs. The details of the preparation of the rubber stoppers will be found in Parts I and II.

Though convenient, the use of a rubber stopper in connection with gas work is not without objection, especially when the experiment is of long duration. An appreciable amount of  $\text{CO}_2$  can be taken up by the rubber (Parts I and II). When utmost accuracy is desirable, it is preferable to have the culture tubes provided with ground glass caps (fig. 2B, Part I) which can be attached to the manometers by means of no. 25 rubber stoppers, or which can be fused on to the tip, thus giving an all-glass connection.

*Culture Jars.*—While the culture tubes when connected with manometers, as just described, were extremely useful in ascertaining the extent of gas exchange taking place under those conditions, they had the disadvantage of a limited capacity. This may be overcome in part by frequent evacuation and refilling with air or oxygen. For many purposes, however, it is preferable to make use of a large air chamber which will be gas-tight and which can be filled with air, or with varying percentages of  $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{N}_2$ , etc. The bottle or jar used for the cultivation of anaerobes is admirably adapted for this type of gas work.

Of the 2 types of Novy jars, the one with a special cock for vacuum work is best adapted for gas studies. The tail end of the cock can be connected with the manometer by means of a no. 25 rubber stopper, while the head is closed with a plugged stopper (fig. 6, Part I). The jar can be used without the manometer.

The organism to be tested was grown on the surface of plates, or in culture tubes,  $20 \times 150$  mm. About 1-5 c.c. of distilled water were placed on the bottom of the jar, or in a tube, in order to provide quickly the necessary aqueous tension. The jar was then sealed in the manner described in Part I. With very little care, the jar can be made perfectly gas-tight. When filled with  $\text{O}_2$  or  $\text{CO}_2$ , or with varying mixtures of gases, and kept at 37 C. for months, no loss in the gas content can be detected.

*Refilling with Air or other Gases.*—In the manipulative work incidental to the study of the gas changes in the culture tube, or in the anaerobe jar, it is necessary to be able to exhaust and to replace the gas contents with fresh air or with varying tensions of  $\text{O}_2$ ,  $\text{CO}_2$  or  $\text{N}_2$ . The full details of the procedure to be followed in such case will be found in Part I.

*The Compensation Manometer.*—In the study of gases, it is essential to be able to observe the pressure changes which take place within the culture tube, or jar, for only in this way is it possible to follow, hour by hour, or day by day, the reaction which takes place. The manometer not only reveals whether an organism is alive and growing, but also indicates the point when growth or respiration ceases. For the description of the manometer, and for details of its use with culture tubes or jars, reference is made to Part I.

*Analyses.*—The determination of the composition of the gas contained in the culture tube or jar, was made by means of Henderson's modification of the Haldane gas apparatus, and is fully described in Part I. At that place will be found, likewise, the method for the estimation of the  $\text{CO}_2$  which is dissolved in the medium.

In the work which follows, it must be recognized that we are observing changes taking place in a definite confined quantity of gas, the initial composition of which is known. The manometric readings show the effect of the metabolic activities of the germs on this gaseous environment, hour by hour, or day by day, until respiration ceases. The analytical values are the quantitative expression of the changes produced by the organisms in this confined gas.

#### RESPIRATION OF *Tr. Lewisi* IN AIR

*Exper. 1.*—Nine *h*-tubes were carefully cleaned. They were first boiled in dilute alkali solution with soap and rinsed with distilled water; then boiled in dilute HCl and again repeatedly washed with water. Finally, they were allowed to dry. Loose cotton plugs were placed in the mouths of the tubes, and the tubes sterilized with dry heat at 200 C. On cooling, 5 cc. of 2% agar were accurately measured into each of the tubes. They were then autoclaved at 110 C., 20 minutes, and allowed to cool in the autoclave to 50 C. Exactly 5 cc. of sterile defibrinated rabbit blood were then added to each tube. The rabbit blood had been kept at 10 C. for 3 days. The tubes were thoroughly shaken to mix the medium, and then were inclined so that the length of the surface of the medium was about 11 cm. The tubes were thus slanted for 12 hours. This gave a soft, juicy medium which retained its shape unless placed in a vertical position.

Seven of the tubes were inoculated each with 1 drop of inoculum. This was spread over the entire surface of the medium with a sterile platinum wire. The 2 uninoculated tubes were similarly treated with sterile wire. Two drops of sterile distilled water were placed in the side-arm of each tube. This water furnished the moisture for the adjustment of the aqueous tension and insured an adequate supply for the growth of the germs.

The cotton plugs in the culture tubes were then cut off, and pushed within the tubes which were now attached to the manometers by means of rubber stoppers treated with glycerol. The stoppers were inserted as firmly as possible into the mouths of the tubes. The stopcocks of the manometers were carefully greased, probed and closed, after which rubber bands were placed around the handles of the cocks to hold them in their seats. The manometers, with the attached tubes, were placed in the hot-room at 29 C., the tubes being supported as shown in fig. 4, Part I.

The water in the side-arm was heated to about 70 C. with a free flame 4 times at intervals of 15 minutes, to adjust rapidly the aqueous tension. After allowing an additional hour for the apparatus to assume the temperature of the hot-room, the stopcocks were opened, and the mercury in the manometers was oscillated by means of a rubber bulb which was applied to cock 1. Cocks 1 and 3 were then closed, and the manometers read to be sure that they were properly equilibrated.

The manometers having been equilibrated, one of the uninoculated tubes was taken for analysis. This analysis was assumed to be representative of the composition of the atmosphere in all of the tubes. It might appear better to analyze each tube before equilibration. This, however, would necessitate the readmittance of air, which would cause a greater error than the assumed constancy of the atmosphere as found by the initial analysis of a control.

The intervals at which the manometers were read was based on the rate of growth, frequent observations being made during the period of rapid multiplication. It had been found that during the first 48 hours there was usually no change in the manometric readings. The gas samples during this interval showed



some, though relatively little, change in composition. The following results taken from an earlier experiment may serve as an example:

|                           |                     |              |              |
|---------------------------|---------------------|--------------|--------------|
|                           | Initial<br>Analysis | 24 hours     | 48 hours     |
| CO <sub>2</sub> per cent. | 0.11                | 1.16         | 1.32         |
| O <sub>2</sub> per cent.  | 20.83               | 19.23        | 18.98        |
|                           | <u>20.94</u>        | <u>20.39</u> | <u>20.30</u> |

This period in the growth of cultures is usually referred to as the "lag" period; its length depends on the activity of the germ. Thus, with the rapidly growing Hay bacillus this period is, as a rule, not over 2 hours in length. Sherman and Albus<sup>10</sup> view this latent period as a biologic rejuvenescence, which,

TABLE 1  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF TR. LEWISI IN AIR

| Tube No. ....         | Cultures   |             |             |             |             |             |             | Uninoculated       |                  |
|-----------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------|------------------|
|                       |            |             |             |             |             |             |             | Initial<br>Control | Final<br>Control |
| Hrs.                  | 1          | 2           | 3           | 4           | 5           | 6           | 7           | 8                  | 9                |
| 0.....                | Mm.<br>0   | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0           | Mm.<br>0         |
| 2.....                | 0          | 0           | 0           | 0           | 0           | 0           | 0           | .....              | 0                |
| 24.....               | 0          | 0           | 0           | 0           | 0           | 0           | 0           | .....              | 0                |
| 48.....               | 0          | 0           | 0           | 0           | 0           | 0           | 0           | .....              | 0                |
| 72.....               | -1         | 0           | 0           | 0           | -1          | 0           | 0           | .....              | 0                |
| 96.....               | -3*        | -2          | -1          | -3          | 3           | -1          | -2          | .....              | 0                |
| 120.....              | .....      | 6           | 4           | 3           | 9           | 3           | 5           | .....              | -1               |
| 144.....              | .....      | -13*        | 12          | 12          | 13          | 9           | 11          | .....              | 2                |
| 168.....              | .....      | .....       | 22          | 20          | 19          | 19          | 22          | .....              | 3                |
| 192.....              | .....      | .....       | 34          | 28          | 23          | 29          | 27          | .....              | 4                |
| 216.....              | .....      | .....       | -34*        | 39          | 35          | 37          | 36          | .....              | 4                |
| 240.....              | .....      | .....       | .....       | 48          | 46          | 45          | 44          | .....              | 4                |
| 264.....              | .....      | .....       | .....       | -51*        | 48          | 45          | 44          | .....              | 4                |
| 288.....              | .....      | .....       | .....       | .....       | -53†        | 46          | 46          | .....              | 4                |
| 312.....              | .....      | .....       | .....       | .....       | .....       | 48          | 46          | .....              | 6                |
| 336.....              | .....      | .....       | .....       | .....       | .....       | -50†        | -46†        | .....              | -8               |
| Analyses at end of... | 96<br>Hrs. | 144<br>Hrs. | 216<br>Hrs. | 264<br>Hrs. | 288<br>Hrs. | 336<br>Hrs. | 336<br>Hrs. | 0<br>Hrs.          | 336<br>Hrs.      |
| CO <sub>2</sub> ..... | 2.04       | 4.71        | 10.08       | 13.46       | 14.05       | 14.99       | 15.05       | 0.21               | 1.43             |
| O <sub>2</sub> .....  | 18.60      | 14.70       | 7.11        | 0.98        | 0.06        | 0.00        | 0.00        | 20.77              | 18.39            |
| Total.....            | 20.64      | 19.41       | 17.19       | 14.44       | 14.11       | 14.99       | 15.05       | 20.98              | 19.82            |

The manometers were equilibrated; barometer, 748 mm.; temperature, 29 C.

\* The cultures were very rich.

† The organisms were granulated.

however, is not the generally accepted interpretation. Some workers prefer to consider it as an expression of an "injury" received by the organism in its previous environment. Wherry and Ervin<sup>11</sup> refer to the interval as a latent period during which CO<sub>2</sub> accumulates to an optimal concentration.

The manometers were read at intervals of 24 hours. Before reading they were given a sharp jerk so as to cause oscillation of the mercury.

<sup>10</sup> Jour. Bacteriol., 1924, 9, p. 303.

<sup>11</sup> Jour. Infect. Dis., 1918, 22, p. 194.

Table 1 shows the manometric readings and the analyses of tubes inoculated with *Tr. lewisi*. It is to be noted that the period of the tests varied from 4 to 14 days. After the analyses, the tubes were removed from the manometers and the growth examined microscopically.

The differences in the rate of development of the negative pressure was due, in part, to the variation in the number of organisms planted. It was obviously impossible to get exact duplicate seedings. Moreover, the capacity of the tubes varied somewhat, and the negative pressure would naturally develop more rapidly in the smaller tubes. The maximal variation in the size of the tubes used in this experiment was 7 c.c.

Before proceeding to discuss the analytical data, the microscopic examinations should be considered. It will be noted that the cultures were exceedingly rich in motile organisms up to the end of the 264th hour. In the next 48 hours, however, marked degeneration took place.

The initial gas content of the tubes was not that of pure air, as shown by the control analysis. The increase in the  $\text{CO}_2$  over that usually found in air was due probably to the liberation of dissolved  $\text{CO}_2$  from the medium.

The rapid development of  $\text{CO}_2$  and the accompanying loss of oxygen, beginning at about the 96th hour, was due to the vigorous multiplication of the culture. The rapid respiration continued until the concentration of oxygen had become considerably reduced.

After the disappearance of the oxygen (288th hour), the  $\text{CO}_2$  continued to increase. This increase, slow as it is, seemed to go on indefinitely. This continued production of  $\text{CO}_2$  in the culture was paralleled by like changes in the uninoculated control tubes. The reaction was clearly a secondary phenomenon due to auto-oxidation and possibly to a progressive decarboxylation of the protein cleavage products. In experiments extending over a period of 6 months, no end to the  $\text{CO}_2$  production was observed.

It is extremely interesting to compare our manometric observations with the course of infection in rats. A rat inoculated with *Tr. lewisi* will show an incubation period of 4 days. The parasites then undergo rapid production for about 6 days, after which the rate of reproduction is retarded, and finally it is inhibited by the 10th day. This inhibition, according to Taliaferro,<sup>12</sup> is due to the formation of a reaction product in the serum which, however, does not kill the organisms.

<sup>12</sup> Footnote 6, p. 139.



Manometrically, the cultures have a "lag" period of 4 days, then rapid reproduction takes place for about 6 days, at which time further growth is inhibited by the lack of oxygen. That the absence of oxygen in the tube is the cause of this inhibition will be shown in exper. 4, in which active growth followed aeration of the culture.

Under the latter conditions, growth, however, does not continue indefinitely. It eventually is arrested though an abundance of oxygen is present. The accumulation of products, whether made directly by the organism or by secondary changes in the medium, brings about cessation of growth and degeneration.

TABLE 1a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 1

| Tube No. ....                           | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
|-----------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Analyses CO <sub>2</sub> .....          | 2.04   | 4.71   | 10.08  | 13.46  | 14.05  | 14.99  | 15.05  |
| O <sub>2</sub> .....                    | 18.60  | 14.70  | 7.11   | 0.96   | 0.06   | 0.00   | 0.00   |
| N <sub>2</sub> .....                    | 20.64  | 19.41  | 17.19  | 14.44  | 14.11  | 14.99  | 15.05  |
|                                         | 79.36  | 80.59  | 82.81  | 85.56  | 85.89  | 85.01  | 84.95  |
| Total.....                              | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Corrected analyses CO <sub>2</sub> .... | 2.03   | 4.61   | 9.62   | 12.27  | 12.92  | 13.93  | 13.99  |
| O <sub>2</sub> ....                     | 18.54  | 14.41  | 6.78   | 0.89   | 0.05   | 0.00   | 0.00   |
| N <sub>2</sub> ....                     | 20.57  | 19.02  | 16.40  | 13.16  | 12.97  | 13.93  | 13.99  |
|                                         | 79.02  | 79.03  | 79.02  | 79.02  | 79.03  | 79.02  | 79.03  |
| Total.....                              | 99.59  | 98.05  | 95.42  | 91.18  | 92.00  | 92.95  | 93.02  |
| Real gain CO <sub>2</sub> .....         | 1.82   | 4.40   | 9.41   | 12.06  | 12.71  | 12.72  | 13.78  |
| Real loss O <sub>2</sub> .....          | 2.23   | 6.36   | 13.99  | 19.88  | 20.72  | 20.77  | 20.77  |
| Real resp. quot. ....                   | 0.816  | 0.691  | 0.672  | 0.606  | 0.613  | 0.612  | 0.663  |
| Calc. manometric readings..             | -2.88  | -14.07 | -32.88 | -56.14 | -57.51 | -49.90 | -50.18 |
| Corr. obs. man. readings....            | -3.04  | -13.36 | -34.6  | -52.0  | -53.8  | -50.7  | -46.7  |

Corrected analyses = analytical value × nitrogen factor, i. e., for tube 1 factor =  $\frac{79.02}{79.36}$

Calculated manometric readings = real loss × (B - aqueous tension)

We may now consider the uninoculated final control. It remained sterile throughout the experiment. It is worthy of note that this tube showed a slowly progressive rise in negative pressure. Analysis revealed an increase in the CO<sub>2</sub> content and a loss of O<sub>2</sub>. The negative pressure was due, in the first place, to the auto-oxidation of the medium, and, secondly, to the solution of CO<sub>2</sub> by the medium and by the rubber stopper. We were unable to determine the consideration to be given this control in our calculations, and for that reason no deductions were made.

The respiratory quotients, as calculated from the analyses, assuming the initial control to be representative for all of the tubes, are given in table 1a.

It is to be noted that the calculated respiratory quotients are low and that they show some variation. These differences, obtained under presumably identical conditions, indicate loss of  $\text{CO}_2$  by solution in the rubber stopper, and even in the medium. That this is true will be demonstrated later when the jar method of determining the respiratory quotient is taken up.

The corrected observed manometric readings were, as a rule, in close concordance with the calculated manometric readings based on the analyses. The greatest difference was that given by tube 4, the calculated reading being 56.1 mm., while the corrected observed reading was 52 mm.

It was assumed, in calculating the readings from analyses, that only  $\text{O}_2$  and  $\text{CO}_2$  underwent a change in concentration. The liberation of small quantities of such substances as  $\text{N}_2$ ,  $\text{NH}_3$  or other products exerting a vapor pressure would influence the observed readings but would not be accounted for in the calculated values.

#### RESPIRATION OF *L. TROPICA* IN AIR

The growth of *L. tropica* on blood agar was such as to be observable macroscopically. On the second day after inoculation, dark round areas appeared on the surface of the medium and seemed to extend quite deep. These spots increased in size until the entire slant had lost its rich red hemoglobin color, which gave way to the formation of the dark brown hematin. At this stage, the surface was covered with a thick, mucus-like, moist, slimy growth, very much like that of a bacterial culture. This surface growth was prominent only when large culture tubes were used. It was due, therefore, to the presence of a relatively large quantity of oxygen.

In the 2 experiments which follow we were able to observe the growth of *L. tropica* over a period of 240 hours. These experiments were performed more than a year apart, and hence cover two different periods in the life of the culture. In exper. 2, the manometers were read at 12 hour intervals, and the tubes were analyzed at 24 hour intervals, beginning with the 24th hour. In exper. 3, the observations were made at 24 hour intervals, and the first analysis was made at the end of the 96th hour.



*Exper. 2.*—Seven *h*-tubes were prepared as already explained. Each of 5 of the tubes received 1 drop of inoculum, which was then spread over the entire surface of the medium with a sterile platinum wire. The 2 uninoculated tubes were treated, in like manner, with the sterile wire in order to have the same exposure to air. Two drops of sterile distilled water were placed in the side-arm of each tube. This water was not heated as we did not appreciate, at that time, the problem of aqueous tension. This water was intended to supply the moisture for the growth of the germ and the needed aqueous tension.

As in the previous experiment, the cotton plugs on the culture tubes were cut off, and the tubes were then attached to manometers using the same procedure as in *exper. 1*.

After allowing 2 hours for the apparatus to assume the temperature of the hot-room, 29 C., the manometers were equilibrated in the manner already given. As before, an initial control analysis was made, and this was assumed to be representative of the composition of the atmosphere in all of the tubes.

*Exper. 3.*—Nine *h*-tubes were prepared as before. The preparation of the medium, the inoculation, and the connection of the tubes with the manometers was carried out exactly as in *exper. 1*. In this experiment, however, the water in the side-arms was heated in order to adjust quickly the aqueous tension. The manometers were equilibrated, after being in the hot-room for 2 hours, and then one of the uninoculated tubes was analyzed as an initial control.

The manometric readings and analyses for these 2 experiments are given in tables 2 and 3.

The difference in the rate of development of the negative pressures in these 2 experiments should be noted. A comparison of the readings in these 2 tables shows that the culture in *exper. 3* was the more active. The manometric readings indicated a consistent rate of growth in the tubes of each experiment, but there was a decided difference in the values given by the two experiments.

This difference is not readily accounted for, but the explanation probably lies in 3 factors, namely, the viability of the germs planted, the absolute number of such organisms inoculated and a possible variation in the mediums used. Certainly, the results in table 3 at the end of 96 hours show much greater activity than is seen at the same time in table 2.

The microscopic examination of each tube, made after sampling, was very interesting. In *exper. 2*, the organisms at the end of 24 hours were not conspicuously active, but they had a healthy appearance.

The other tubes examined after 48, 72, 96 and 120 hours were exceedingly rich with masses of perfect flagellates, showing no indications of unfavorable conditions. In *exper. 3*, there was the same richness of growth in tubes 1, 2 and 3. The next tube examined (168 hours) showed a marked difference; the organisms were granulated and slow. Examinations of tubes 5, 6, 7 (192, 216 and 240 hours) showed extreme granulation of the germs with only now and then a motile cell. A study of the analyses reveals the fact that the  $O_2$  was practically gone at the end of 144 hours and that the organisms were subjected to the action of about 14% of  $CO_2$ .

It was observed in all of these test-tube experiments that the cultures were rich and that the organisms were extremely active, though the tension of  $CO_2$  was greatly increased, while that of  $O_2$  was considerably decreased as compared with the tension of these gases in the blood.

TABLE 2  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN AIR

| Tube No. ....           | Cultures |         |         |         |          | Uninoculated  |                 |
|-------------------------|----------|---------|---------|---------|----------|---------------|-----------------|
|                         | 1        | 2       | 3       | 4       | 5        | Final Control | Initial Control |
| Hrs. ....               | 0        | 0       | 0       | 0       | 0        | 0             | 0               |
| 12.....                 | 0        | 0       | 0       | 0       | 0        | 0             | ....            |
| 24.....                 | -2*      | -1      | -4      | -4      | -2       | -1            | ....            |
| 36.....                 | ....     | 4       | 6       | 5       | 7        | 1             | ....            |
| 48.....                 | ....     | -9*     | 9       | 11      | 18       | 2             | ....            |
| 72.....                 | ....     | ....    | -22*    | 23      | 28       | 2             | ....            |
| 96.....                 | ....     | ....    | ....    | -28*    | 39       | 2             | ....            |
| 120.....                | ....     | ....    | ....    | ....    | -39*     | -4            | ....            |
| Analyses at end of..... | 24 Hrs.  | 48 Hrs. | 72 Hrs. | 96 Hrs. | 120 Hrs. | 120 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 1.89     | 4.03    | 4.69    | 8.33    | 10.70    | 1.67          | 0.14            |
| O <sub>2</sub> .....    | 18.76    | 15.23   | 14.36   | 8.86    | 5.22     | 19.12         | 20.80           |
| Total.....              | 20.65    | 19.26   | 19.05   | 17.19   | 15.92    | 20.79         | 20.94           |

The manometers were equilibrated at a barometric pressure of 756 mm., temperature 30.5 C.  
\* The cultures were very rich.

TABLE 2a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 2

| Tube No. ....                        | 1      | 2      | 3      | 4      | 5      |
|--------------------------------------|--------|--------|--------|--------|--------|
| Analyses CO <sub>2</sub> .....       | 1.89   | 4.03   | 4.69   | 8.33   | 10.70  |
| O <sub>2</sub> .....                 | 18.76  | 15.23  | 14.36  | 8.86   | 5.22   |
| N <sub>2</sub> .....                 | 20.65  | 19.26  | 19.05  | 17.19  | 15.92  |
| Total.....                           | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Corr. analyses CO <sub>2</sub> ..... | 1.88   | 3.94   | 4.58   | 7.95   | 10.00  |
| O <sub>2</sub> .....                 | 18.69  | 14.91  | 14.02  | 8.45   | 4.90   |
| N <sub>2</sub> .....                 | 20.57  | 18.85  | 18.60  | 16.40  | 14.90  |
| Total.....                           | 99.63  | 97.91  | 97.66  | 95.46  | 94.02  |
| Real gain CO <sub>2</sub> .....      | 1.74   | 3.80   | 4.44   | 7.81   | 9.92   |
| Real loss O <sub>2</sub> .....       | 2.11   | 5.89   | 5.78   | 11.55  | 15.90  |
| Respiratory quotient.....            | 0.824  | 0.645  | 0.768  | 0.676  | 0.624  |
| Calc. man. reading.....              | -2.67  | -15.18 | -15.9  | -32.8  | -43.24 |
| Corr. obs. man. reading.....         | -2.03  | -9.15  | -22.35 | -28.7  | -39.66 |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric reading = real loss × (B - aqueous tension).

TABLE 3  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN AIR

| Tube No. ....         | Cultures   |             |             |             |             |             |             | Uninoculated    |               |
|-----------------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|---------------|
|                       |            |             |             |             |             |             |             | Initial Control | Final Control |
|                       | 1          | 2           | 3           | 4           | 5           | 6           | 7           | 8               | 9             |
| Hrs. ....             | Mm.        | Mm.         | Mm.         | Mm.         | Mm.         | Mm.         | Mm.         | Mm.             | Mm.           |
| 0.....                | 0          | 0           | 0           | 0           | 0           | 0           | 0           | 0               | 0             |
| 24.....               | 0          | 0           | 0           | 0           | 0           | 0           | 0           | ....            | 0             |
| 48.....               | -10        | -10         | -3          | -5          | -11         | -9          | -3          | ....            | 0             |
| 72.....               | 31         | 24          | 16          | 15          | 20          | 28          | 15          | ....            | 0             |
| 96.....               | -47*       | 38          | 83          | 27          | 32          | 32          | 26          | ....            | 0             |
| 120.....              | ....       | -49*        | 46          | 39          | 41          | 42          | 37          | ....            | 0             |
| 144.....              | ....       | ....        | -52*        | 51          | 45          | 49          | 47          | ....            | -1            |
| 168.....              | ....       | ....        | ....        | -55†        | 53          | 49          | 48          | ....            | 2             |
| 192.....              | ....       | ....        | ....        | ....        | -53†        | 51          | 46          | ....            | 3             |
| 216.....              | ....       | ....        | ....        | ....        | ....        | -51†        | 46          | ....            | 4             |
| 240.....              | ....       | ....        | ....        | ....        | ....        | ....        | -46†        | ....            | 4             |
| 336.....              | ....       | ....        | ....        | ....        | ....        | ....        | ....        | ....            | -8            |
| Analyses at end of... | 96<br>Hrs. | 120<br>Hrs. | 144<br>Hrs. | 168<br>Hrs. | 192<br>Hrs. | 216<br>Hrs. | 240<br>Hrs. | 0<br>Hrs.       | 336<br>Hrs.   |
| CO <sub>2</sub> ..... | 11.52      | 10.41       | 14.70       | 13.16       | 13.91       | 14.15       | 15.33       | 0.21            | 1.43          |
| O <sub>2</sub> .....  | 4.08       | 5.87        | 0.00        | 1.61        | 1.06        | 1.03        | 0.00        | 20.76           | 18.39         |
| Total.....            | 15.60      | 16.28       | 14.70       | 14.67       | 14.99       | 15.18       | 15.33       | 20.97           | 19.82         |

The manometers were equilibrated, the barometric pressure being 748 mm., and the temperature 29.8 O.

\* The cultures were very rich.

† The organisms were nearly or completely granulated.

TABLE 3a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 3

| Tube No. ....                            | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
|------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Analyses CO <sub>2</sub> .....           | 11.52  | 10.42  | 14.70  | 13.16  | 13.91  | 14.15  | 15.33  |
| O <sub>2</sub> .....                     | 4.08   | 5.87   | 0.00   | 1.51   | 1.08   | 1.03   | 0.00   |
| N <sub>2</sub> .....                     | 15.60  | 16.29  | 14.70  | 14.67  | 14.99  | 15.18  | 15.33  |
|                                          | 84.40  | 83.71  | 85.30  | 85.33  | 85.01  | 84.82  | 84.67  |
| Total.....                               | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Corrected analyses CO <sub>2</sub> ..... | 10.78  | 9.83   | 13.61  | 12.19  | 12.93  | 13.18  | 14.31  |
| O <sub>2</sub> .....                     | 3.80   | 5.54   | 0.00   | 1.40   | 1.03   | 0.96   | 0.00   |
| N <sub>2</sub> .....                     | 14.58  | 15.37  | 13.61  | 13.59  | 13.96  | 14.14  | 14.31  |
|                                          | 79.05  | 79.03  | 79.04  | 79.02  | 79.00  | 79.03  | 79.03  |
| Total.....                               | 98.63  | 94.40  | 92.65  | 92.61  | 92.96  | 93.17  | 93.34  |
| Real gain CO <sub>2</sub> .....          | 10.57  | 9.62   | 13.40  | 11.96  | 12.72  | 12.97  | 13.35  |
| Real loss O <sub>2</sub> .....           | 16.96  | 15.22  | 20.76  | 19.36  | 19.73  | 19.80  | 20.76  |
| Resp. quotient.....                      | 0.620  | 0.632  | 0.645  | 0.614  | 0.644  | 0.655  | 0.643  |
| Calc. man. readings.....                 | -45.79 | -40.19 | -52.7  | -52.3  | -50.22 | -48.9  | -54.56 |
| Corr. obs. man. readings....             | -47.51 | -49.6  | -52.7  | -55.88 | -53.9  | -51.46 | -46.6  |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric readings = real loss × (B - aqueous tension)



A comparison of tables 1 and 3 shows a striking difference in the rate of growth of the 2 organisms. These 2 tables lend themselves very well to comparison, since blood from the same animal was used in the preparation of the mediums. In time, both organisms consumed all of the oxygen. This was accomplished by *L. tropica* in one-half of the time required by *Tr. lewisi*. The more rapid and more luxuriant growth of the former accounted for this difference.

Tables 2*a* and 3*a* show the calculated respiratory quotients and calculated manometric readings for the cultures just described. The quotients in table 2*a* vary somewhat, from 0.624 to 0.824, while in table 3*a* the differences are not so great (0.614-0.655), though the values are low for the reasons mentioned in connection with table 1*a*.

A comparison of the calculated and observed manometric readings, as given in either table 2*a* or 3*a*, shows more or less variation. This is due in part to the difficulty of securing an exact control analysis of the air which is present in each tube at the start of the experiment. In addition to this, if the aqueous tension is not fully established at the time of equilibration, it will result in a lower observed reading than is deduced from the analysis.

It was assumed in the early part of this work that when water was placed in the side-arm, the aqueous tension would adjust itself during the 2 hours previous to equilibration. This, however, is not the case, for a positive pressure of 1-5 mm. was often observed after equilibration, clearly due to the gradual adjustment of vapor pressure. It therefore became evident that the adjustment of aqueous tension could not be secured in this way. Hence, in connection with the tests given in table 3*a*, an effort was made to obviate this by heating the water contained in the side-arm. This, it will be seen, resulted in manometric readings which, with one exception, were somewhat higher than those calculated.

The same treatment applied to the tubes given in table 1*a* produced fairly concordant results, the variation being less than 4 mm.

#### FULLY DEVELOPED CULTURES OF *TR. LEWISI* AND *L. TROPICA* IN AIR

At the end of the "lag" period in the previous experiments, as indicated, the germs were undergoing very rapid reproduction. It is evident that observations made during this interval necessarily represent changes due to organisms which are undergoing little or no multiplication. The analytical values obtained during the period of active reproduction which continues until the maximal negative pressure is

reached, are representative of a young and rapidly growing culture. It is unlikely that the respiratory quotient during this interval is different from that at any other time. The following experiment was intended to bring out the changes produced by cultures which were fully developed and undergoing rapid multiplication. On account of the large number of organisms present, it was expected that the respiration process would begin and end earlier than it did when the tubes were freshly inoculated.

TABLE 4  
SHOWING, IN THE FIRST AERATION TEST, THE RAPIDITY IN RISE OF NEGATIVE PRESSURE WITH THE FINAL RESULTS OF ANALYSES

| Tube 4, Exper. 3<br><i>L. tropica</i>                              |     | Tube 4, Exper. 1<br><i>Tr. lewisi</i> |     |
|--------------------------------------------------------------------|-----|---------------------------------------|-----|
| Hrs.                                                               | Mm. | Hrs.                                  | Mm. |
| 0.....                                                             | 0   | 0.....                                | 0   |
| 4.....                                                             | 0   | 2.....                                | 0   |
| 8.....                                                             | -1  | 7.....                                | 0   |
| 18.....                                                            | 10  | 9.....                                | -1  |
| 23.....                                                            | 12  | 20.....                               | 7   |
| 43.....                                                            | 25  | 28.....                               | 10  |
| 49.....                                                            | 28  | 33.....                               | 20  |
| 54.....                                                            | 30  | 45.....                               | 24  |
| 56.....                                                            | 31  | 57.....                               | 30  |
| 66.....                                                            | 38  | 70.....                               | 40  |
| 74.....                                                            | 39  | 78.....                               | 44  |
| 90.....                                                            | 46  | 97.....                               | 51  |
| 102.....                                                           | 49  | 117.....                              | 57  |
| 114.....                                                           | 56  | 141.....                              | -64 |
| 138.....                                                           | 62  |                                       | —   |
| 162.....                                                           | -62 |                                       | —   |
| Then analyzed they gave % CO <sub>2</sub> .....                    |     | 12.96                                 |     |
| O <sub>2</sub> .....                                               |     | 0.10                                  |     |
|                                                                    |     | 13.06                                 |     |
| The same tubes in the initial test gave a maximal pressure of..... |     | -51                                   |     |
|                                                                    |     | —51                                   |     |
| In                                                                 |     | 168 Hrs.                              |     |
| And analysis gave CO <sub>2</sub> .....                            |     | 264 Hrs.                              |     |
| O <sub>2</sub> .....                                               |     | 13.46                                 |     |
|                                                                    |     | 0.98                                  |     |
|                                                                    |     | 14.44                                 |     |

*Exper. 4.*—Several of the tubes from exper. 1 and 3 were taken. The tubes after analysis were removed from the manometers, and a specimen was taken with a platinum loop for microscopic examination. The cotton plugs were put loosely in the tubes which were then left to aerate by diffusion. The bright red hemoglobin color returned to the medium as the CO<sub>2</sub> traveled outward. The color in the *Tr. lewisi* tubes became as bright as when originally planted; the *L. tropica* tubes seemed to be darker as though some permanent reaction had taken place in the medium. After 24 hours of diffusion, to make sure that all of the gas in the culture tubes was replaced with normal air, a glass tube was introduced, and filtered air was blown through for 5 minutes. The side-arms were also thoroughly aerated. This method of aeration was employed in this experiment because it had been necessary to remove the tubes from the manometers for the microscopic examination.

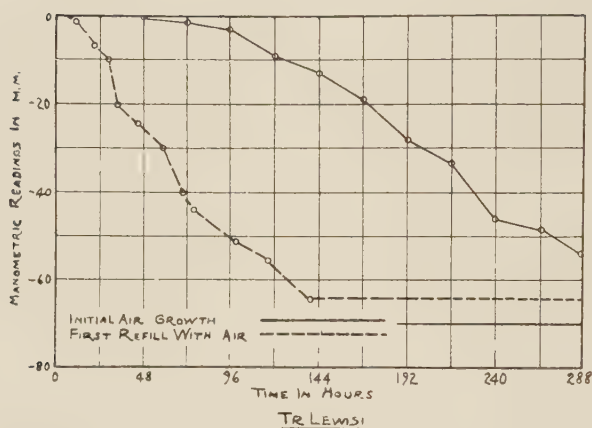


Chart 1.—Showing the rapidity in development of negative pressure of an initial culture and the same culture in the first aeration. *Tr. Lewisii*.

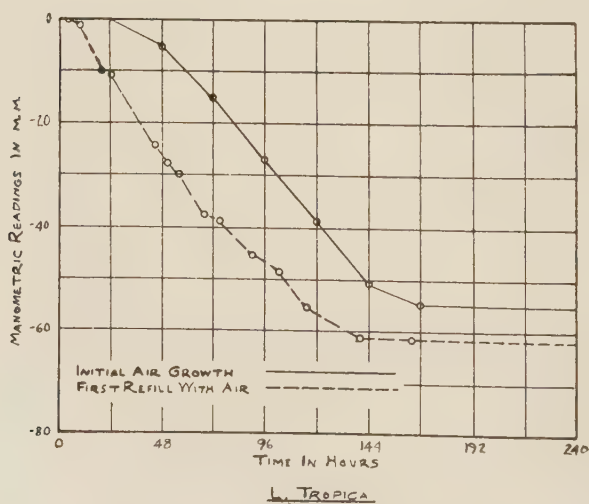


Chart 2.—Showing the rapidity in development of negative pressure of an initial culture and the same culture in the first aeration. *L. tropica*.



The tubes were then reattached to the manometers in the usual manner, returned to 29 C., and after an interval of 2 hours, they were equilibrated, and cocks 1 and 3 were closed. The aeration of the culture tubes was effected without the slightest evidence of contamination.

The results of this experiment for 1 tube each of *L. tropica* and *Tr. lewisi* are given in table 4, and are also presented graphically in charts 1 and 2. The culture of the former, as indicated in table 3, was highly granular, while that of the latter was very rich in good cells (table 1).

It should be pointed out that the yield of  $\text{CO}_2$  in these tests was lower than that obtained in the first trials. This was probably due to some fixation of  $\text{O}_2$  by the reducing products present in the more or less disintegrating mass of cells.

It will be seen that the negative pressure manifested itself earlier than at the start of the other experiments. Likewise, it will be seen that the previous maximum was reached in a shorter time.

The manometric response, as seen in table 4, indicated that a distinct "lag" period was present, although it was shorter than in exper. 1 and 2. It was possibly due to the age and condition of the cultures employed. This was contrary to what might be expected with an active and well developed culture. Consequently, the following experiment was made to show the absence of such a "lag" period in a young and well developed culture of *L. tropica*.

*Exper. 5.*—Four side-arm tubes (fig. 2C, Part I) were carefully cleaned and dried. Tight cotton plugs were placed in the mouths of the tubes and a loose pledget in the side-arm. The tubes were sterilized at 200 C. On cooling, 5 c.c. of 2% agar were measured into each tube. They were then autoclaved at 110 C. for 20 minutes; cooled in the autoclave to 50 C., after which 5 c.c. of sterile defibrinated rabbit blood were added to each tube. The rabbit blood had been kept at 10 C. for 48 hours. The tubes were shaken and slanted so that the length of the surface of the medium was about 10 cm. The tubes were left in this position for 12 hours.

Two of the tubes were inoculated as usual. The 2 control tubes were also treated with the sterile platinum wire. The mouth of each tube, after removal of the cotton plug, was now sealed in the blast lamp, and then each tube was sealed to the end of a manometer, thus avoiding all rubber connections. The actual air capacity of these tubes was thus reduced to about 30 c.c. The manometers were placed at 29 C., and equilibrated after 2 hours. One of the uninoculated tubes (No. 3) was then analyzed as the initial control.

The culture tubes were analyzed at the end of 95 hours when it seemed certain that the maximal negative pressure had been reached. The manometers with the 2 inoculated tubes were then attached by means of cocks 3 to a glass rake, which connected with the vacuumeter, and the tubes were evacuated to —715 mm., after which air was admitted through a tube filled with sterile cotton.

This operation was repeated 5 times so as to replace all the gas in the culture tubes with normal air. The manometers were then equilibrated, and cocks 1 and 3 were closed. Tube 1 was analyzed as an initial control. The results of this experiment are given in table 5.

It will be seen that in tube 2, after aeration, the negative pressure began to appear at once, and that the maximum was reached in a somewhat shorter time than before. Thus, it was evident that no

TABLE 5  
SHOWING, IN THE FIRST AERATION TEST, THE ABSENCE OF A "LAG" PERIOD. *L. TROPICA*

| Tube No. ....  | Cultures |     | Uninoculated    |               |
|----------------|----------|-----|-----------------|---------------|
|                |          |     | Initial Control | Final Control |
|                | 1        | 2   | 3               | 4             |
| Hrs.           | Mm.      | Mm. | Mm.             | Mm.           |
| 0*.....        | 0        | 0   | 0               | 0             |
| 15.....        | -2       | 0   | ....            | 0             |
| 29.....        | 3        | 0   | ....            | 0             |
| 41.....        | 8        | -4  | ....            | 0             |
| 53.....        | 19       | 17  | ....            | 0             |
| 65.....        | 33       | 39  | ....            | 0             |
| 77.....        | 47       | 57  | ....            | -1            |
| 89.....        | 57       | 59  | ....            | 2             |
| 95.....        | -57      | -59 | ....            | -2            |
| First aeration |          |     |                 |               |
| 0†.....        | 0        | 0   |                 |               |
| 1.....         | ....     | -1  |                 |               |
| 2.....         | ....     | 2   |                 |               |
| 3.....         | ....     | 3   |                 |               |
| 4.....         | ....     | 4   |                 |               |
| 5.....         | ....     | 5   |                 |               |
| 6.....         | ....     | 6   |                 |               |
| 7.....         | ....     | 7   |                 |               |
| 8.....         | ....     | 8   |                 |               |
| 14.....        | ....     | 14  |                 |               |
| 26.....        | ....     | 29  |                 |               |
| 38.....        | ....     | 42  |                 |               |
| 52.....        | ....     | 51  |                 |               |
| 62.....        | ....     | -54 |                 |               |

\* The manometers were equilibrated, the barometer at 750 mm., temperature 29 C.

† The manometers were equilibrated, the barometer at 748 mm., temperature 29 C.

"lag" period was present when a fully developed, active culture was employed. It should be added that this culture, at the time of aeration, was presumably very rich and in good condition, whereas in the older culture of *L. tropica* which was used in exper. 4, the organisms were nearly or completely granulated as indicated in table 3.

#### *L. TROPICA* UNDER INCREASED OXYGEN TENSION

Shortly after the isolation of oxygen, observers found that respiration of the gas produced inflammatory effects. No appreciable change

in the elimination of  $\text{CO}_2$  was observed by Regnault and Reiset.<sup>13</sup> Later, Lorrain Smith<sup>14</sup> found that mice subjected to partial pressures of 73.6-79.9% of oxygen succumbed in 3-4 days. More recently, Moore and Williams<sup>15</sup> found that the tubercle bacillus grew badly or not at all in concentrations of about 80% of an atmosphere of oxygen. Adams,<sup>16</sup> extending the work of these 2 investigators, found only 2 organisms out of 26 tested to be oxyphobic, the tubercle bacillus and *B. pestis*. Concentrations of 60% or more were toxic for the plague bacillus, but only inhibitive for the tubercle bacillus. He also studied the effect of high oxygen tensions on mammals.

The use of  $\text{O}_2$  in the treatment of disease makes the study of its effects on the pathogenic organisms of special importance. It might be assumed that parasitic organisms are adapted to low tensions of  $\text{CO}_2$  and  $\text{O}_2$ , such as exist in the tissues, and that an appreciable change in the concentration of these gases in the culture tubes would inhibit their growth. It was therefore with the object of determining the influence of high tensions of  $\text{O}_2$  that the following experiments were made.

It should be pointed out before proceeding with the experimental work, that when the germs are planted on a solid medium, it does not follow that they will all remain on such surface. It is possible, especially with a fluid inoculum and a soft medium, for some of the organisms to penetrate along the glass wall, and even through the medium itself, into the deeper layers. Such organisms, sheltered from the direct effect of the higher concentrations, may proceed to prepare conditions which are favorable to those remaining on the surface. Irregularity in the manometric readings, in parallel tubes, as seen in tables 12 and 13, may be due to this factor.

The manipulative work necessary in connection with the introduction of different gas tensions into culture tubes or jars has been given in detail in Part I. Consequently, only an outline of the procedure is given below.

In each of the experiments which follow, 2 control tubes were inoculated at the same time, closed with rubber caps and incubated at the same temperature as the others. At the end of 4 or 5 days, they were examined. In every instance, a rich growth was obtained thus eliminating any question as to the viability of the germs planted.

<sup>13</sup> Ann. d. Chim. et d. Phys. (3), 1849, 26, p. 299.

<sup>14</sup> Jour. Physiol., 1899, 24, p. 19.

<sup>15</sup> Bio-Chem. Jour., 1909, 4, p. 177; 1911, 5, p. 181.

<sup>16</sup> Ibid., 1912, 6, p. 297.



*Growth in 30% Oxygen; Exper. 6.*—Four *h*-tubes were prepared in the usual way. Two of the tubes were inoculated with 1 drop of the rich culture fluid, and the inoculum was subsequently spread with a sterile platinum wire. The 2 uninoculated tubes were also treated with the sterile wire. The loose cotton plugs were cut off and pushed into the tubes, which were then attached to the manometers by means of rubber stoppers treated with glycerol. The manometers were then connected by cocks 3 to a glass rake, which in turn was joined to the special T connector shown in fig. 8, Part I. The latter was attached to the suction apparatus and to the O<sub>2</sub> and N<sub>2</sub> supply.

With the necessary precaution as to the manometers, the tubes were evacuated to —700 mm., and then N<sub>2</sub> was admitted.

This operation was repeated 5 times, allowing 10 minutes between exhaustions for diffusion of the gases. Finally, the tubes were evacuated to —225 mm., and, after replacing the N<sub>2</sub> in the connecting line with O<sub>2</sub>, the latter was admitted into the tubes until the mercury in the manometers indicated the zero level. The no. 3 cocks were then closed and the manometers disconnected from the rake and placed in the hot-room. After 2 hours, they were equilibrated with the tip of stop-cock 3 below the level of water in a small beaker.

After equilibration, one of the tubes was analyzed and was found to have 31.7% of O<sub>2</sub>.

The reason why a higher value was obtained than was contemplated was due to the fact that the uncorrected barometric pressure was used in the calculation. The barometric pressure was 744 mm., and since the aqueous tension at 31 C. is 33.7 mm., it follows that  $B - T = 710.3$ , and 30% of this equals 213, whereas actually the evacuation was carried to —223 mm.

It should be mentioned here that in all of the work in which the gas to be determined was more than 25% of the sample, it was necessary to take less than 3 c.c., for the reason that on the gas buret, though of 10 c.c. capacity, only the lower 3 c.c. were graduated. To obtain this small sample, it was necessary first to measure out approximately 7 c.c. of nitrogen, which was then transferred into the pyrogallate tube. About 3 c.c. of the sample were then drawn into the buret, the nitrogen was then brought over, and the total volume read.

The limit of accuracy therefore in these experiments was less than when a 10 c.c. sample was used. Extreme instances of such error can be seen in the control tubes in tables 6 and 7.

It will be seen from table 6, which contains the results of this experiment, that a pressure of —96 mm. was reached in about 192 hours, and that the analyses showed the O<sub>2</sub> to be reduced to 0.13 and 0.91%, while the CO<sub>2</sub> content rose to more than 20%.

The increase of oxygen tension to 30% seemed to have a stimulative effect on the growth of the organism, as indicated by the rapid fall in the pressure. The maximal negative pressure was obtained when the O<sub>2</sub> was nearly gone. This point was reached in 192 hours, or in about the same time as when air was present (table 3). It is to be noted that while, in the latter, the maximal pressure was about —53 mm., in this experiment, it rose to —96 mm. In tables 7 and 8, the pressure became considerably higher. This increase in the negative pressure is a necessary consequence of an increase in the amount of O<sub>2</sub> consumed, as has been pointed out in Part I.

*Growth in 40% O<sub>2</sub>; Exper. 7.*—The procedure in this experiment was the same as in the preceding one, except that a higher concentration of O<sub>2</sub> was used. The results will be found in table 7. It is worthy of note that the negative

TABLE 6  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 30% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
|                         | 1        | 2        | 3             | 4               |
| Hrs.                    | Mm.      | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0        | 0        | 0             | 0               |
| 24.....                 | -2       | -2       | 0             | ....            |
| 48.....                 | 22       | 8        | 0             | ....            |
| 72.....                 | 41       | 23       | -1            | ....            |
| 96.....                 | 57       | 40       | 2             | ....            |
| 120.....                | 74       | 56       | 2             | ....            |
| 144.....                | 85       | 70       | 2             | ....            |
| 168.....                | 96       | 84       | 2             | ....            |
| 192.....                | -97*     | 95       | 2             | ....            |
| 216.....                | ....     | -96*     | -2            | ....            |
| Analyses at end of..... | 192 Hrs. | 216 Hrs. | 216 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 21.38    | 20.27    | 0.79          | 0.15            |
| O <sub>2</sub> .....    | 0.13     | 0.91     | 26.47         | 31.47           |
|                         | 21.51    | 21.18    | 27.26         | 31.62           |

The manometers were equilibrated with the barometer reading 744 mm. and the temperature 31 C.

\* The organisms were granulated.

TABLE 7  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 40% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
|                         | 1        | 2        | 3             | 4               |
| Hrs.                    | Mm.      | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0        | 0        | 0             | 0               |
| 24.....                 | -4       | -2       | 0             | ....            |
| 48.....                 | 11       | 13       | 0             | ....            |
| 72.....                 | 29       | 43       | -2            | ....            |
| 96.....                 | 45       | 64       | 2             | ....            |
| 120.....                | 57       | 78       | 2             | ....            |
| 144.....                | 68       | 92       | 2             | ....            |
| 168.....                | 79       | 104      | 2             | ....            |
| 192.....                | 88       | 113      | 2             | ....            |
| 216.....                | 110      | 117      | 2             | ....            |
| 240.....                | -110*    | -117*    | -2            | ....            |
| Analyses at end of..... | 240 Hrs. | 240 Hrs. | 240 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 25.99    | 26.18    | 0.85          | 0.63            |
| O <sub>2</sub> .....    | 3.23     | 0.00     | 41.72         | 37.94           |
| Total.....              | 29.27    | 26.18    | 42.57         | 38.67           |

The manometers were equilibrated at a barometric pressure of 744 mm. and a temperature of 31 C.

\* The organisms were granulated.

pressure reached was higher than when 30% O<sub>2</sub> was used, being —110 and —117 mm., respectively. Analysis showed the presence of 26% CO<sub>2</sub>, and in one tube (no. 2) an entire absence of O<sub>2</sub>. Clearly, this higher content of O<sub>2</sub> stimulates rather than retards the growth of this organism.

*Growth in 50% O<sub>2</sub>; Exper. 8.*—The manometric readings and analytical data are given in table 8. The growth of the organisms in this atmosphere was luxuriant. Macroscopic examination showed a heavy surface growth. The ability of the germ to grow in this concentration of O<sub>2</sub> and produce such a high partial pressure of CO<sub>2</sub> was extremely interesting, since, as will be shown later the use of 30% of CO<sub>2</sub> does not permit the growth of the organism in a freshly inoculated tube.

TABLE 8  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 50% OXYGEN

| Tube No. ....           | Cultures |         | Uninoculated  |                 |
|-------------------------|----------|---------|---------------|-----------------|
|                         |          |         | Final Control | Initial Control |
| Hrs. ....               | 1        | 2       | 3             | 4               |
| 0.....                  | Mm. 0    | Mm. 0   | Mm. 0         | Mm. 0           |
| 24.....                 | —3       | —3      | 0             | ....            |
| 48.....                 | 15       | 16      | 0             | ....            |
| 72.....                 | 40       | 30      | 0             | ....            |
| 96.....                 | 65       | 51      | 0             | ....            |
| 120.....                | 91       | 67      | —2            | ....            |
| 144.....                | 108      | 76      | 2             | ....            |
| 168.....                | 126      | 89      | 2             | ....            |
| 192.....                | 143      | 98      | 2             | ....            |
| 216.....                | 152      | 102     | 2             | ....            |
| 240.....                | 154      | 111     | 6             | ....            |
| 264.....                | 155      | 116     | 6             | ....            |
| 288.....                | 156      | 122     | 6             | ....            |
| 312.....                | —157*    | 126     | 10            | ....            |
| 336.....                | ....     | 130     | —10           | ....            |
| 360.....                | ....     | 134     | ....          | ....            |
| 408.....                | ....     | —137*   | ....          | ....            |
| Analyses at end of..... | 312 Hrs. | 408 Hrs | 312 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 33.38    | 33.88   | 1.34          | 0.07            |
| O <sub>2</sub> .....    | 0.09     | 0.11    | 47.23         | 48.19           |
| Total.....              | 33.47    | 33.99   | 48.58         | 48.26           |

The manometers were equilibrated at a barometric pressure of 748 mm. and temperature of 32 C.

\* The organisms were granulated.

It is to be noted in the table that the O<sub>2</sub> had practically disappeared and was replaced by more than 33% of CO<sub>2</sub>. Further, that pressures of —137 and —157 were attained. Recalculation of the results gave 0.692 and 0.703 as the respective real respiratory quotients of the 2 cultures. These values, however, are not correct largely because of loss of CO<sub>2</sub> by solution in the rubber stopper and in the medium. Exact determinations of the respiratory quotient, by means of the jar method, will be given later.

*Growth in 60% O<sub>2</sub>; Exper. 9.*—The data for this experiment are given in table 9. The early manometric readings gave the impression that the germs were going to utilize this high concentration of O<sub>2</sub>. But the readings gradually slowed



down and reached a maximum long before the  $O_2$  was entirely consumed. It will be seen that a maximal pressure of only—88 mm. was reached, which is markedly in contrast with —137 to —157 mm. obtained with 50%  $O_2$  in table 8. Microscopic examination showed the organisms to be granulated, or, more correctly, completely disintegrated. The development of the culture had therefore reached an end when only about 20%  $CO_2$  was formed, and while there was still 30 or more % of  $O_2$  present. This result finds confirmation in the following experiments and is in striking contrast with that obtained in the preceding trial. It could hardly be expected that an increment of 10% would exert so marked an effect. The fact, however, remains that 60%  $O_2$  is definitely inhibitive.

TABLE 9  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 60% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
| Hrs.                    | 1        | 2        | 3             | 4               |
| 0.....                  | Mm.<br>0 | Mm.<br>0 | Mm.<br>0      | Mm.<br>0        |
| 24.....                 | 0        | 0        | 0             | .....           |
| 48.....                 | —5       | —5       | 0             | .....           |
| 72.....                 | 13       | 13       | —2            | .....           |
| 96.....                 | 20       | 26       | 2             | .....           |
| 120.....                | 32       | 38       | 4             | .....           |
| 144.....                | 42       | 51       | 6             | .....           |
| 168.....                | 53       | 61       | 6             | .....           |
| 192.....                | 60       | 68       | 6             | .....           |
| 216.....                | 66       | 73       | 6             | .....           |
| 240.....                | 72       | 80       | 8             | .....           |
| 264.....                | 88       | 84       | 8             | .....           |
| 288.....                | —88*     | 88       | 8             | .....           |
| 312.....                | .....    | —88*     | —8            | .....           |
| Analyses at end of..... | 288 Hrs. | 312 Hrs. | 312 Hrs.      | 0 Hrs.          |
| $CO_2$ .....            | 21.75    | 19.62    | 1.57          | 0.32            |
| $O_2$ .....             | 29.98    | 37.05    | 60.68         | 62.68           |
| Total.....              | 61.73    | 56.67    | 62.25         | 62.90           |

The manometers were equilibrated at a barometric pressure of 740 mm. and a temperature of 31° C.

\* The organisms were granulated.

Incidentally, it may be mentioned that at this concentration of oxygen Adams<sup>10</sup> found that *B. pestis* was killed.

*Growth in 80%  $O_2$ ; Exper. 10.*—The data are given in table 10. The inhibition noted in the previous experiment was now more marked. No surface growth was present, and, on final examination, though some organisms were found, they were completely disintegrated. The slight respiration observed was probably the result of protected growth in the medium, to which attention has already been called. It will suffice to point out that, in this experiment, the yield of  $CO_2$  was only 7%, while the unused  $O_2$  approximated 68%, and that at the same time the pressures reached only —42 mm. and —53 mm., respectively.

*Growth in 100%  $O_2$ ; Exper. 11.*—The results of this test are given in table 11. No growth was observable macroscopically in the medium. Clearly, under this

TABLE 10  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 80% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
| Hrs.                    | Mm.      | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0        | 0        | 0             | 0               |
| 24.....                 | 0        | 0        | 0             | ....            |
| 48.....                 | -2       | -3       | 0             | ....            |
| 72.....                 | 6        | 5        | 0             | ....            |
| 96.....                 | 7        | 11       | -10           | ....            |
| 120.....                | 16       | 17       | 12            | ....            |
| 144.....                | 19       | 27       | 12            | ....            |
| 168.....                | 24       | 30       | 13            | ....            |
| 192.....                | 27       | 36       | 13            | ....            |
| 216.....                | 31       | 41       | 13            | ....            |
| 240.....                | 35       | 44       | 14            | ....            |
| 264.....                | 37       | 48       | 14            | ....            |
| 288.....                | 39       | 51       | 14            | ....            |
| 312.....                | 41       | 53       | -14           | ....            |
| 336.....                | -42*     | -53*     | ....          | ....            |
| Analyses at end of..... | 336 Hrs. | 336 Hrs. | 312 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 7.05     | 7.57     | 2.42          | 0.44            |
| O <sub>2</sub> .....    | 67.86    | 68.31    | 73.41         | 80.23           |
| Total.....              | 74.91    | 75.88    | 75.83         | 80.57           |

The manometers were equilibrated at a barometric pressure of 748 mm. and a temperature of 33 C.

\* The organisms were granulated.

TABLE 11  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *L. TROPICA* IN 100% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
| Hrs.                    | Mm.      | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0        | 0        | 0             | 0               |
| 24.....                 | 0        | 0        | 0             | ....            |
| 48.....                 | 0        | 0        | 0             | ....            |
| 66.....                 | -1       | 0        | 0             | ....            |
| 90.....                 | 2        | 0        | 0             | ....            |
| 114.....                | 3        | 0        | 0             | ....            |
| 144.....                | 3        | 0        | 0             | ....            |
| 175.....                | 5        | 0        | 0             | ....            |
| 195.....                | 5        | 0        | 0             | ....            |
| 211.....                | -5*      | 0*       | 0             | ....            |
| Analyses at end of..... | 211 Hrs. | 211 Hrs. | 211 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 1.15     | 1.23     | 0.87          | 0.35            |
| O <sub>2</sub> .....    | 97.65    | 96.53    | 97.65         | 96.16           |
| Total.....              | 98.80    | 97.76    | 98.52         | 96.51           |

The manometers were equilibrated with a barometric pressure of 749 mm. and a temperature of 31 C.

\* The organisms were granulated.

extreme concentration of  $O_2$ , there was really no evidence of multiplication, since the manometers registered little or no change, and the analyses confirmed this fact. The small amount of  $CO_2$  formed was undoubtedly due to cadaveric respiration.

*Discussion of Results in Exper. 6-11.*—The growth, and hence the respiration, of *L. tropica* was apparently stimulated by partial pressures of  $O_2$  under 60%. But when the tension reached 60%, a distinctly injurious effect was noted, which became more and more marked as the concentration of  $O_2$  was increased.

Just how far the volume of the gas in these tubes influenced the result, it is not possible to state, since no tests were made with anaerobic jars which have a 20-fold capacity. It is conceivable, however, that in tubes which as in this case have a capacity of about 100 c.c., the injurious effect is held back, if not done away with entirely, at certain concentrations, as, for example, 50%, by the early reduction of the relatively small volume of  $O_2$  to a concentration that has little or no effect. Be that as it may, the limiting point appears to be reached, for the 100 c.c. volume, when the  $O_2$  concentration is 60%.

Just how the high tensions of  $O_2$  produce these striking results, it is not possible to state. It may be supposed that the oxidative changes within the cells are increased to a point at which the vitality of the organism becomes exhausted. This may imply inhibition or even destruction of the respiratory enzyme; or, it may mean the formation of oxidation products which are directly injurious to the cells. Certainly, the presence of  $CO_2$  does not seem to counteract the effect due to such high  $O_2$  tensions.

#### TR. LEWISI UNDER INCREASED OXYGEN TENSION

Unlike *L. tropica*, this organism rarely gives rise to a visible growth in air, and the organisms are never as numerous. And yet, when grown in air, *Tr. lewisi* consumes all of the  $O_2$  within the culture tube almost as rapidly as does *L. tropica*. The latter, as shown by tube 7 in table 3, removed all of the  $O_2$  within 240 hours, while the former practically accomplished the same result in 288 hours (tube 5, table 1). It might be expected from this that the 2 organisms would behave about alike under increased  $O_2$  tension. Actually, however, *Tr. lewisi* appears to be more sensitive than *L. tropica* to an increase in the concentration of oxygen. Thus, while the latter has been shown to grow vigorously in 50%  $O_2$ , the former is markedly inhibited in a

somewhat lower concentration. It would have been of interest to compare the behavior of cultures of *Tr. lewisi* in air with those in 25, 30, 35 and 40% O<sub>2</sub>, but such tests were not made.

*Growth in 45% O<sub>2</sub>; Exper. 12.*—The details for this and the subsequent experiment were the same as those observed in the preceding tests. The inoculum as usual was a very rich one. As can be seen on reference to table 12, the negative pressure developed rather slowly, which in itself was conclusive evidence that the organisms were not growing as rapidly as under ordinary air conditions (table 1). Therefore, on analysis, it was expected that a large quantity of O<sub>2</sub>

TABLE 12  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *TR. LEWISI* IN 45% OXYGEN

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
| Hrs. ....               | 1        | 2        | 3             | 4               |
| 0.....                  | Mm.<br>0 | Mm.<br>0 | Mm.<br>0      | Mm.<br>0        |
| 24.....                 | 0        | 0        | 0             | .....           |
| 48.....                 | 0        | 0        | 0             | .....           |
| 72.....                 | -1       | -2       | 0             | .....           |
| 96.....                 | 4        | 4        | 0             | .....           |
| 120.....                | 8        | 6        | 0             | .....           |
| 144.....                | 12       | 10       | 0             | .....           |
| 168.....                | 16       | 13       | -1            | .....           |
| 192.....                | 20       | 16       | 2             | .....           |
| 216.....                | 24       | 18       | 4             | .....           |
| 240.....                | 26       | 19       | 6             | .....           |
| 264.....                | 30       | 21       | 8             | .....           |
| 288.....                | 32       | 23       | 11            | .....           |
| 312.....                | 35       | 24       | 11            | .....           |
| 336.....                | 38       | 26       | 11            | .....           |
| 360.....                | 40       | 26       | 14            | .....           |
| 384.....                | -43*     | -27*     | -17           | .....           |
| Analyses at end of..... | 284 Hrs. | 284 Hrs. | 284 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 5.45     | 2.96     | 0.85          | 0.11            |
| O <sub>2</sub> .....    | 34.85    | 40.80    | 41.77         | 46.23           |
| Total.....              | 40.30    | 43.76    | 42.62         | 46.34           |

The manometers were equilibrated with a barometric reading of 744 mm. and a temperature of 30 C.

\* The organisms were granulated.

and a small amount of CO<sub>2</sub> would be found, and such was actually the case. A concentration of O<sub>2</sub>, which is about twice that of ordinary air, is therefore clearly inhibitive.

An examination of the cultures at the end of 384 hours showed that they were granulated, but it was by no means certain that they were dead. To test this point, the cultures were aerated and then incubated for 5 days. A very rich growth developed, which indicated a mere inhibition on the part of the O<sub>2</sub>. As is well known, *Tr. lewisi* possesses considerable vitality, and this explains its subsequent development under the more favorable condition, that of the O<sub>2</sub> tension in ordinary air.



*Growth in 60% O<sub>2</sub>; Exper. 13.*—The growth of the organism was again inhibited, as could be expected from the previous experiment. The culture, however, was viable at the end of 456 hours, since subsequent aeration and incubation gave a rich growth. A comparison of the manometric readings for the 2 inoculated cultures (table 13) shows an appreciable difference in the final pressures, though a similar difference is also to be noted in table 12.

These two experiments with *Tr. lewisi* are in striking agreement with those of *L. tropica*. It is evident that increased oxygen tension is distinctly inhibitive, if not injurious to these organisms. This is all the more remarkable considering the hardness of the organisms and

TABLE 13  
MANOMETRIC READINGS AND ANALYSES OF GROWTH OF *TR. LEWISI* IN 60% O<sub>2</sub>

| Tube No. ....           | Cultures |          | Uninoculated  |                 |
|-------------------------|----------|----------|---------------|-----------------|
|                         |          |          | Final Control | Initial Control |
|                         | 1        | 2        | 3             | 4               |
| Hrs. ....               | Mm.      | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0        | 0        | 0             | 0               |
| 14.....                 | —3       | 0        | 0             | —               |
| 72.....                 | 8        | —8       | —1            | ....            |
| 96.....                 | 10       | 9        | 1             | ....            |
| 144.....                | 15       | 13       | 3             | ....            |
| 168.....                | 18       | 14       | 3             | ....            |
| 192.....                | 20       | 15       | 4             | ....            |
| 240.....                | 26       | 18       | 5             | ....            |
| 288.....                | 30       | 19       | 6             | ....            |
| 312.....                | 34       | —21*     | 6             | ....            |
| 408.....                | 40       | ....     | 7             | ....            |
| 456.....                | —44*     | ....     | —8            | ....            |
| Analyses at end of..... | 456 Hrs. | 312 Hrs. | 456 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 6.55     | 1.85     | 1.63          | 0.00            |
| O <sub>2</sub> .....    | 52.32    | 57.73    | 58.95         | 60.52           |
| Total.....              | 58.87    | 59.58    | 60.58         | 60.52           |

The manometers were equilibrated at barometric pressure of 743 mm. and the temperature 30 C.

\* The organisms were granulated.

the vigor of growth which they show under ordinary air conditions. It will be seen that the former is somewhat more sensitive than the latter.

#### TR. LEWISI UNDER INCREASED CO<sub>2</sub> TENSION

It was pointed out in connection with exper. 1 that the microscopic examination made after the maximal negative pressure had been reached showed extreme granulations of the germs. At this stage the gaseous atmosphere contained about 15% of CO<sub>2</sub> and no O<sub>2</sub>. Since the toxic effect of CO<sub>2</sub> on some protozoa has been reported by many observers,

it was desirable to establish whether the degeneration noted above was due to the increase in  $\text{CO}_2$  or to the removal of the  $\text{O}_2$ . With this object in view, the following experiments were made with atmospheres containing variable amounts of  $\text{CO}_2$  and an abundant supply of  $\text{O}_2$ .

*Exper. 14.*—Twelve tubes (20 x 150 mm.) received the usual medium, and were closed with loose cotton plugs. After inoculation, the plugs were cut off and pushed within the tubes. Two of these tubes and 5 drops of  $\text{H}_2\text{O}$  were then placed in each of 6 anaerobic jars of about 2,000 c.c. capacity.

The jars were sealed in the usual manner, but were not attached to manometers. Jars 2 to 6 were then evacuated and filled with varying concentrations

TABLE 14  
EFFECTS OF VARYING CONCENTRATIONS OF  $\text{CO}_2$  ON *Tr. LEWISI*, 2 TUBES IN EACH JAR,  
3 WEEKS AT 31 C.

| Jar.....                            | 1     | 2     | 3      | 4      | 5      | 6     |
|-------------------------------------|-------|-------|--------|--------|--------|-------|
| Initial analyses $\text{CO}_2$ .... | 0.03  | 6.93  | 22.58  | 30.58  | 38.54  | 50.80 |
| $\text{O}_2$ ....                   | 20.92 | 19.41 | 13.27  | 14.39  | 12.84  | 10.51 |
| Final analyses $\text{CO}_2$ ....   | 2.91  | 10.36 | 21.51  | 31.69  | 39.35  | 51.00 |
| $\text{O}_2$ ....                   | 17.55 | 15.47 | 13.91* | 13.30* | 11.90* | 9.69* |
| Growth.....                         | Rich  | Rich  | None   | None   | None   | None  |

\* The organisms showed extreme granulation.

TABLE 15  
EFFECT OF VARYING CONCENTRATIONS OF  $\text{CO}_2$  ON *L. TROPICA*, 2 TUBES IN EACH JAR,  
3 WEEKS AT 31 C.

| Jar                 | 1     | 2     | 3     | 4      |
|---------------------|-------|-------|-------|--------|
| Initial analyses    |       |       |       |        |
| $\text{CO}_2$ ..... | 0.21  | 9.57  | 20.40 | 29.02  |
| $\text{O}_2$ .....  | 20.51 | 19.24 | 16.64 | 14.58  |
| Final analyses      |       |       |       |        |
| $\text{CO}_2$ ..... | 2.92  | 13.54 | 23.55 | 30.30  |
| $\text{O}_2$ .....  | 17.61 | 14.76 | 13.08 | 11.29* |
| Growth.....         | Rich  | Rich  | Rich  | None   |

\* The organisms showed extreme granulation.

of  $\text{CO}_2$ , after which they were placed in the hot-room for 2 hours. Jar 1 contained ordinary air and served as a control. The gaseous content of each jar was then sampled and analyzed. To relieve the excess pressure which had developed within the jars because of the increased temperature, the stop-cock was momentarily opened with the tip of the cock under water. After 3 weeks' incubation at 31 C., the jars were again sampled and then opened for microscopic examination. The data are given in table 14.

On reference to table 14, it will be seen that  $\text{CO}_2$  in a concentration of 20% or more possessed a decidedly toxic or inhibitive effect on the growth of *Tr. lewisi*. The final analyses of jars 3-6 showed practically no gas exchange, and this was confirmed by the microscopic

examination of the cultures, which revealed no evidence of multiplication. On the other hand, the tubes in jars 1 and 2 showed rich growths, and the analyses indicated an appreciable gas exchange. It is to be noted that the inhibitive effect of  $\text{CO}_2$  was manifested in the presence of an abundance of oxygen.

#### L. TROPICA UNDER INCREASED $\text{CO}_2$ TENSION

*Exper. 15.*—Four jars, each containing 2 tubes which were inoculated with *L. tropica*, were used in this experiment. Jar 1 contained ordinary air and served as a control. Jars 2 to 4 were evacuated and filled with varying concentrations of  $\text{CO}_2$ . They were then placed in the hot-room at 31 C., and, after 2 hours, the gas content of each jar was determined. As in the previous experiment, at the end of 3 weeks, the content of each jar was again analyzed. The data are given in table 15.

As could be expected from the previous experiment,  $\text{CO}_2$  was found to be toxic for *L. tropica* even though plenty of  $\text{O}_2$  was present. The difference in sensitivity of the 2 germs, already noted in the  $\text{O}_2$  experiments, was again conspicuous. Thus, in jar 3, *L. tropica* produced a more marked gas exchange than did *Tr. lewisi* in the corresponding jar. It may be well to point out that in jar 2, in which the initial  $\text{CO}_2$  tension was less than 10%, the gaseous exchange was apparently more marked than in the corresponding air controls. It would therefore appear that this amount of  $\text{CO}_2$  possessed a stimulating action. Before accepting this interpretation, it would be necessary to know the exact air capacity of each jar.

In the case of *L. tropica*, this favoring action was also seen in jar 3, in which there was 20% of  $\text{CO}_2$ . It is noteworthy that this concentration, which was toxic for *Tr. lewisi*, was not injurious to *L. tropica*. The  $\text{CO}_2$ , however, began to show its unfavorable action in jar 4, in which its concentration was nearly 30%. There was no growth in this jar, whereas in the other 3 rich cultures were developed. *L. tropica* was, therefore, found to be less sensitive to increased tensions of  $\text{CO}_2$  and of  $\text{O}_2$  than was *Tr. lewisi*.

*Tr. lewisi and L. tropica in 100%  $\text{CO}_2$ ; Exper. 16.*—This experiment was made before those given under nos. 14 and 15; and hence, before it had been learned that  $\text{CO}_2$ , in 20 or 30% concentration sufficed to inhibit one or the other of these organisms. It follows, therefore, that no growth could be expected in this extreme concentration. The results of this experiment are presented here because of the interesting facts revealed.

It will be seen from table 16 that 8 tubes were used. These were *h*-tubes and contained the usual medium. After being attached to manometers, they were filled with 100%  $\text{CO}_2$ , and were then placed in the hot-room at 31 C. Uninoculated tube 7 was analyzed, as an initial control, and showed over 98%  $\text{CO}_2$ . The data are given in table 16.

The point especially to be noted is that the tubes showed a rapid rise in negative pressure, which, under ordinary air or oxygen conditions, would have indicated a very good growth. But it will be seen that control tube 8 developed the same gain in negative pressure.

The fact that the atmosphere consisted wholly or almost so of  $\text{CO}_2$  excluded any growth other than anaerobic. But, in such case, a marked positive pressure would be expected. Here, however, there was a progressive negative rise which was not at an end even in 204 hours. Clearly, no organism could be expected to bring about such

TABLE 16  
MANOMETRIC READINGS OF CULTURES OF *Tr. LEWISI* AND *L. TROPICA* GROWN IN 100%  $\text{CO}_2$

| Tube No. ....           | Tr. Lewisi |      |      | L. tropica |       |       | Uninoculated    |               |
|-------------------------|------------|------|------|------------|-------|-------|-----------------|---------------|
|                         |            |      |      |            |       |       | Initial Control | Final Control |
| Hrs.                    | Mm.        | Mm.  | Mm.  | Mm.        | Mm.   | Mm.   | Mm.             | Mm.           |
| 0.....                  | 0          | 0    | 0    | 0          | 0     | 0     | 0               | 0             |
| 2.....                  | -8         | -12  | -9   | -14        | -7    | -12   | ....            | -10           |
| 4.....                  | 14         | 20   | 14   | 19         | 15    | 19    | ....            | 18            |
| 6.....                  | 21         | 28   | 20   | 23         | 23    | 26    | ....            | 23            |
| 8.....                  | 27         | 32   | 27   | 29         | 29    | 31    | ....            | 29            |
| 10.....                 | 33         | 37   | 31   | 35         | 33    | 38    | ....            | 34            |
| 12.....                 | 40         | 43   | 38   | 37         | 37    | 48    | ....            | 38            |
| 36.....                 | 55         | 60   | 51   | 58         | 55    | 62    | ....            | 59            |
| 60.....                 | 61         | 65   | 58   | 65         | 63    | 68    | ....            | 67            |
| 84.....                 | 69         | 69   | 65   | 70         | 71    | 77    | ....            | 73            |
| 108.....                | 74         | 73   | 69   | 74         | 77    | 85    | ....            | 79            |
| 132.....                | 80         | 76   | 71   | 79         | 83    | 90    | ....            | 82            |
| 156.....                | 84         | 81   | 75   | 86         | 88    | 94    | ....            | 88            |
| 180.....                | 89         | 84   | 80   | 90         | 92    | 100   | ....            | 92            |
| 204.....                | -93        | -88† | -83† | -97        | -101† | -107† | ....            | -98           |
| Analyses at end of..... | 204 Hrs.   | .... | .... | 204 Hrs.   | ....  | ....  | 0 Hrs.          | 204 Hrs.      |
| $\text{CO}_2$ .....     | 98.46      | .... | .... | 98.54      | ....  | ....  | 98.16           | 98.08         |
| $\text{O}_2$ .....      | 0.38       | .... | .... | 0.14       | ....  | ....  | 0.38            | 0.46          |
| Total.....              | 98.46      | .... | .... | 98.68      | ....  | ....  | 98.54           | 98.54         |

The manometers were equilibrated at a barometric pressure of 744 mm. and temperature of 31 C.

† Not analyzed.

consumption of  $\text{CO}_2$ . The cause for this steady loss of  $\text{CO}_2$  had to be sought elsewhere. One factor, without doubt, was the medium which would absorb a sufficient amount until full saturation was reached. The continued rise of the negative pressure pointed, however, to some other cause.

It was shown in Part I of this series that finely cut rubber rapidly absorbed  $\text{CO}_2$  out of an atmosphere containing 100% of this gas. Hence, the chief cause for the peculiar manometric behavior in this case was the absorption or solution of  $\text{CO}_2$  in the rubber stopper.



It may be well to add that on subsequent aeration of the culture tubes no growth resulted; hence, the  $\text{CO}_2$  in this concentration was toxic for the 2 organisms. It will be shown later that these organisms are not destroyed by exposure to atmospheres of  $\text{N}_2$  or of  $\text{H}_2$ .

The failure of the organisms to grow after aeration, in the previous experiment, suggested the possibility that some reaction product of the acid gas and the medium was the toxic agent. The dark color of the medium might indicate the formation of such a substance. To test the point, it was desirable, therefore, to submit tubes of mediums to varying concentrations of  $\text{CO}_2$  previous to inoculation.

TABLE 17

THE MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *L. TROPICA* GROWN ON MEDIUMS PREVIOUSLY EXPOSED TO PARTIAL PRESSURES OF 10, 20 AND 100%  $\text{CO}_2$

| Per cent.....           | 10       |          | 20       |          | 100      |          |
|-------------------------|----------|----------|----------|----------|----------|----------|
| Tube No. ....           | 1        | 2        | 3        | 4        | 5        | 6        |
| Hrs.                    | Mm.      | Mm.      | Mm.      | Mm.      | Mm.      | Mm.      |
| 0.....                  | 0        | 0        | 0        | 0        | 0        | 0        |
| 24.....                 | -4       | -4       | -5       | -6       | -2       | -2       |
| 48.....                 | 20       | 18       | 17       | 20       | 18       | 15       |
| 67.....                 | 36       | 36       | 33       | 38       | 31       | 29       |
| 91.....                 | 50       | 48       | 47       | 50       | 37       | 43       |
| 115.....                | 51       | 48       | 50       | 51       | 37       | 47       |
| 143.....                | -51*     | -51*     | -51*     | -52*     | -37*     | -47*     |
| Analyses at end of..... | 143 Hrs. | 143 Hrs. | 143 Hrs. | 143 Hrs. | 143 Hrs. | 143 Hrs. |
| $\text{CO}_2$ .....     | 14.00    | 14.43    | 14.28    | 14.27    | 16.21    | 14.86    |
| $\text{O}_2$ .....      | 0.13     | 0.13     | 0.09     | 0.00     | 0.26     | 0.33     |
|                         | 14.13    | 14.56    | 14.37    | 14.27    | 16.47    | 15.19    |

The manometers were equilibrated, the barometer reading 748 mm., the temperature 31 C.

\* The organisms were granulated.

*L. tropica* on Mediums Previously Exposed to  $\text{CO}_2$ ; *Exper. 17*.—Nine *h*-tubes were prepared with the usual blood-agar medium. The uninoculated tubes were attached to manometers, which were then connected, 3 at a time, to the vacuumeter by means of the glass rake. The 3 sets were then filled with 10, 20 and 100%  $\text{CO}_2$ , respectively, after which the tubes were disconnected, and a control from each set was taken for analysis. The remaining 6 manometers, with the attached tubes, were then placed in the hot-room and incubated for 5 days.

At the end of that period the medium in all of the tubes was dark. The manometers were then removed from the hot-room; the tubes were taken off, and, after loosening the cotton plugs, they were allowed to aerate, by diffusion, for 12 hours. Finally, to insure complete aeration, filtered air was blown over the medium and through the side-arm of each tube.

The tubes were now inoculated with *L. tropica* in the usual way. They were then reattached to the manometers and placed at 31 C. and, after 2 hours, they were equilibrated. The manometric readings and analyses are given in table 17.

The manometric readings indicated vigorous growth and multiplication, the maximal negative pressure being reached in about 91 hours, which was somewhat earlier than in exper. 2 and 3. The analyses indicated that the  $O_2$  was practically gone, and that from 14-16% of  $CO_2$  was present. This experiment conclusively showed that, under these conditions no injurious compound was formed, or, at least, it was not retained in the medium which had been saturated with the varying tensions of  $CO_2$ . Further, it was evident that the destruction of the organisms, noted in the previous experiments, was due to the high concentration of  $CO_2$  employed.

TR. LEWISI AND L. TROPICA IN THE ABSENCE OF  $CO_2$

The previous experiments, together with those which will be presented later, show that  $O_2$  is necessary for the growth of these organisms in the culture tube. Consequently, following the classification of Pasteur,<sup>17</sup> they are obligative aerobes. During the period of growth carbonic acid is returned in a definite ratio to the oxygen consumed. Waste product though it is, conceivably, its presence may be necessary for the aerobic growth of these organisms. From that standpoint, it was desirable to ascertain whether or not  $CO_2$  was essential for the development of cultures of these protozoa.

The removal of all of the  $CO_2$  from a vigorously growing culture, as fast as it is formed, is extremely difficult. In their work with the tubercle bacillus, Moore and Williams<sup>15</sup> placed their culture tubes under bell-jars along with soda-lime to absorb the  $CO_2$  which was formed. While, according to their analyses, no  $CO_2$  was present in the air of the bell-jar, it was not certain that the culture tubes, which were closed with cotton plugs, did not have some  $CO_2$  present. Similar experiments by Adams<sup>16</sup> showed a slow but retarded growth of the tubercle bacillus, and this fact in itself was direct evidence that  $CO_2$  was being made. The outward diffusion of this gas from the bottom of a narrow test-tube, where it is being formed, proceeds very slowly as is to be expected from the density of the gas. Therefore, in order to remove the  $CO_2$  as fast as it is produced, the absorbing reagent must be placed in close proximity to the source of gas production.

In view of the results presented in Part II and of the work mentioned, it was desirable to study the growth of these protozoa in the

<sup>17</sup> Compt. rend. Acad. Sc., 1863, 56, p. 1192.

total absence of  $\text{CO}_2$ , and, as a preliminary test of this kind, the following experiments with tube cultures were made.

*Tr. lewisi* in the Apparent Absence of  $\text{CO}_2$ ; *Exper. 18*.—Nine *h*-tubes were prepared each with 5 cc. of agar. After autoclaving, 5 cc. of sterile 10% KOH solution were pipetted into the side-arms. The defibrinated rabbit blood was then added as usual to the agar and the mediums slanted. The tubes were then inoculated, and the cotton plugs, after having been cut off, were pushed down so as in no wise to obstruct the opening to the side-arm. The tubes were now attached to manometers and placed in the hot-room and

TABLE 18  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *TR. LEWISI* GROWN IN TUBES WITH 5 C.C. 10% KOH IN THE SIDE-ARM

| Tube No. ....         | Cultures    |             |             |             |             |             |             | Uninoculated    |               |
|-----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|---------------|
|                       |             |             |             |             |             |             |             | Initial Control | Final Control |
|                       | 1           | 2           | 3           | 4           | 5           | 6           | 7           | 8               | 9             |
| Hrs. ....             | Mm.         | Mm.         | Mm.         | Mm.         | Mm.         | Mm.         | Mm.         | Mm.             | Mm.           |
| 0.....                | 0           | 0           | 0           | 0           | 0           | 0           | 0           | 0               | 0             |
| 36.....               | -7          | -5          | -5          | -5          | -5          | -5          | -5          | ....            | -7            |
| 60.....               | 11          | 8           | 7           | 8           | 8           | 7           | 8           | ....            | 7             |
| 88.....               | 24          | 13          | 11          | 14          | 12          | 8           | 10          | ....            | 8             |
| 109.....              | 40          | 22          | 19          | 22          | 19          | 14          | 16          | ....            | 8             |
| 140.....              | 66          | 36          | 30          | 33          | 35          | 24          | 22          | ....            | 13            |
| 153.....              | 84          | 50          | 42          | 45          | 48          | 30          | 32          | ....            | 13            |
| 180.....              | 111         | 72          | 62          | 62          | 70          | 42          | 44          | ....            | 15            |
| 207.....              | 141         | 106         | 93          | 83          | 95          | 60          | 68          | ....            | 18            |
| 231.....              | 141         | 125         | 112         | 98          | 108         | 70          | 80          | ....            | 26            |
| 251.....              | -141*       | -146*       | 131         | 136         | 122         | 88          | 95          | ....            | 26            |
| 299.....              | ....        | ....        | -143†       | 138         | 140         | 100         | 122         | ....            | 26            |
| 346.....              | ....        | ....        | ....        | -145†       | 140         | 100         | 125         | ....            | 30            |
| 369.....              | ....        | ....        | ....        | ....        | -140†       | 110         | 128         | ....            | -30           |
| 465.....              | ....        | ....        | ....        | ....        | ....        | -120†       | -133†       | ....            | ....          |
| Analyses at end of... | 251<br>Hrs. | 251<br>Hrs. | 299<br>Hrs. | 346<br>Hrs. | 369<br>Hrs. | 921<br>Hrs. | 465<br>Hrs. | 0<br>Hrs.       | 369<br>Hrs.   |
| $\text{CO}_2$ .....   | 0.00        | 0.00        | 0.00        | 0.00        | 0.00        | 0.00        | 0.00        | 0.00            | 0.00          |
| $\text{O}_2$ .....    | 0.00        | 0.33        | 0.60        | 0.21        | 0.08        | 1.89        | 1.75        | 20.89           | 18.10         |
| Total.....            | 0.00        | 0.33        | 0.60        | 0.21        | 0.08        | 1.89        | 1.75        | 20.89           | 18.10         |

The manometers were equilibrated with the barometer reading 748 mm., the temperature 31 C.

\* Cultures showed considerable degeneration.

† The organisms were completely granulated.

equilibrated as usual. It will be evident from table 18 that no inhibition of growth took place, since rich cultures developed in the tubes. It is to be noted that the maximal negative pressure was nearly 3 times as great as that in *exper. 1*. The organisms consumed all or nearly all of the  $\text{O}_2$  and produced  $\text{CO}_2$ , which, however, was absorbed by the KOH, thus causing the high negative reading. The latter closely approximated the partial pressure of  $\text{O}_2$  originally present in the air.

The analytical results show that no  $\text{CO}_2$  was present, but the analyses were made after the maximal negative pressure was reached,

and hence were no index of the presence or absence of  $\text{CO}_2$  during the period of rapid growth. This condition was taken into account in the next experiment.

*L. tropica* in the Apparent Absence of  $\text{CO}_2$ ; *Exper. 19*.—The previous experiment was repeated using *L. tropica* as the inoculum. The manometric readings and analyses are given in table 19. The microscopic examinations which were made during the period of rapid growth (tubes 1-4) showed the organisms to be in good form and exceedingly active, notwithstanding the fact that the

TABLE 19  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *L. TROPICA* GROWN IN TUBES WITH  
5 C.C. 10% KOH IN THE SIDE-ARM

| Tube No. ....         | Cultures   |            |            |             |             |             |             | Uninoculated       |                  |
|-----------------------|------------|------------|------------|-------------|-------------|-------------|-------------|--------------------|------------------|
|                       |            |            |            |             |             |             |             | Initial<br>Control | Final<br>Control |
| Hrs. ....             | 1          | 2          | 3          | 4           | 5           | 6           | 7           | 8                  | 9                |
| 0.....                | Mm.<br>0   | Mm.<br>0   | Mm.<br>0   | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0    | Mm.<br>0           | Mm.<br>0         |
| 24.....               | -14        | -4         | -9         | -4          | -3          | -7          | -4          | ....               | 0                |
| 48.....               | -41*       | 31         | 39         | 30          | 8           | 35          | 18          | ....               | 0                |
| 60.....               | ....       | 55         | 55         | 47          | 18          | 49          | 33          | ....               | -5               |
| 72.....               | ....       | -67*       | 66         | 57          | 24          | 62          | 40          | ....               | 7                |
| 84.....               | ....       | ....       | 78         | 70          | 35          | 75          | 52          | ....               | 8                |
| 96.....               | ....       | ....       | -90*       | 85          | 41          | 88          | 65          | ....               | 8                |
| 110.....              | ....       | ....       | ....       | 97          | 58          | 99          | 77          | ....               | 10               |
| 120.....              | ....       | ....       | ....       | -109*       | 68          | 105         | 87          | ....               | 9                |
| 145.....              | ....       | ....       | ....       | ....        | 86          | 116         | 104         | ....               | 12               |
| 169.....              | ....       | ....       | ....       | ....        | 98          | 122         | 118         | ....               | 13               |
| 190.....              | ....       | ....       | ....       | ....        | 107         | 127         | 129         | ....               | 15               |
| 217.....              | ....       | ....       | ....       | ....        | 114         | 129         | 135         | ....               | 17               |
| 302.....              | ....       | ....       | ....       | ....        | -120†       | -130†       | 140         | ....               | 20               |
| 352.....              | ....       | ....       | ....       | ....        | ....        | ....        | -140†       | ....               | -24              |
| Analyses at end of... | 48<br>Hrs. | 72<br>Hrs. | 96<br>Hrs. | 120<br>Hrs. | 302<br>Hrs. | 302<br>Hrs. | 352<br>Hrs. | 0<br>Hrs.          | 352<br>Hrs.      |
| $\text{CO}_2$ .....   | 0.10       | 0.12       | 0.04       | 0.00        | 0.00        | 0.00        | 0.00        | 0.00               | 0.00             |
| $\text{O}_2$ .....    | 15.31      | 11.77      | 9.03       | 5.16        | 3.79        | 2.81        | 0.00        | 20.90              | 17.66            |
| Total.....            | 15.41      | 11.89      | 9.07       | 5.16        | 3.79        | 2.81        | 0.00        | 20.90              | 17.66            |

The manometers were equilibrated with barometer reading 746 mm., and the temperature at 30 C.

\* The cultures were rich with well-shaped organisms.

† The organisms were completely granulated.

analyses revealed little or no  $\text{CO}_2$ . The medium in this as in the previous experiment retained its rich red color. This was the more noticeable since the ordinary cultures of *L. tropica* were characterized by marked changes in color.

In the previous experiment, no  $\text{CO}_2$  was revealed by the analyses because the examinations were all made when the cultures had reached their full development. It was indicated in that connection that  $\text{CO}_2$  could have been found had analyses been made during the period of



active multiplication. This experiment furnished definite proof of this fact. It will be seen on reference to table 18 that tubes 1-3 contained a small amount of  $\text{CO}_2$ , while the subsequent cultures had none. Obviously, during the period of rapid respiration the  $\text{CO}_2$  cannot be instantaneously absorbed; hence, a small residuum is demonstrable. It would be wrong to assume that the culture developed in the entire absence of  $\text{CO}_2$ .

In the 2 preceding experiments, it was shown that the organisms had grown perfectly in the tubes, although alkali was present in the side-arm. It was clear that the removal of the  $\text{CO}_2$  was not as complete or as rapid as was desired. It seemed that possibly the removal could be facilitated by employing a partial vacuum in connection with the alkali, and accordingly the following experiment was made.

*L. tropica* in *h*-tubes with 10% KOH in the Side-Arm, 10%  $\text{O}_2$  Tension and a pressure of  $-180$  mm.; *Exper. 20*.—Seven *h*-tubes were prepared as usual, and the medium slanted; 5 c.c. of 10% KOH were then placed in the side-arm of 6 of them, and 5 c.c. of  $\text{H}_2\text{O}$  were similarly placed in the 7th tube. Five of the tubes, including the water tube, were inoculated as usual. The other 2 tubes were kept as uninoculated controls. The tubes were then attached to the manometers which were connected by cocks 3 to a glass rake, evacuated to  $-350$  mm., and refilled with nitrogen to zero reading, after which they were placed at 30 C. Later, they were reconnected with the vacuumeter in the hot-room and evacuated to  $-180$  mm., thus creating in the tubes a diminished pressure, approximating 10%, which it was hoped would facilitate the removal of the  $\text{CO}_2$ .

The data are recorded in table 20. Minute dark, round areas, indicating growth, were observed in the medium at the end of 24 hours. The appearance of the germs in tube 1, at the end of 72 hours, was excellent, but the tube again showed the presence of a small amount of  $\text{CO}_2$ .

Clearly, the  $\text{CO}_2$  which was produced by an actively growing culture was not instantly removed by the alkali which was present in the adjoining side-arm. The amount of this gas, however, was reduced to a small fraction of 1%. The favoring action of  $\text{CO}_2$ , if such there was, would therefore rest with traces, rather than with any high percentage, of this gas.

The other tubes, including no. 5, the water tube, when examined 42 hours later, had a fairly rich growth, but were granulated. It will be seen from the analysis of tube 2 that no  $\text{CO}_2$  was present. On the other hand, tube 5, which contained no alkali, had the full amount of  $\text{CO}_2$  which could be expected considering the diminished  $\text{O}_2$  tension originally present.

The experiment just given failed to realize the expectation as to complete and rapid removal of  $\text{CO}_2$ . Accordingly, the method of

procedure was modified by resorting to the use of Petri dishes in the manner given in the following experiment, whereby the alkali was brought into the closest possible proximity to the culture.

*L. tropica* in Petri Dishes Over 5% KOH; Exper. 20 A.—In this and the following experiments a 3% agar was employed in order to have as firm a medium as possible. Each of 6 ordinary tubes received 5 c.c. of agar; after autoclaving, an equal volume of sterile, defibrinated rabbit blood was added, and the contents were then poured into sterile Petri dishes. The dishes were placed at 34 C. for 12 hours in order to set as firmly as possible. Four drops of inoculum were then placed on the blood agar in each plate, and the material was spread evenly over the surface by means of a sterile bent glass rod. Four of the plates were then inverted over 4 plates of the same diameter containing 10 c.c. of KOH. The distance between the blood agar surface and that of the alkali was less than 1 cm. Four narrow strips of adhesive tape

TABLE 20  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *L. TROPICA* IN TUBES WITH 10% O<sub>2</sub> TENSION, 5 C.C. 10% KOH IN SIDE-ARM AND INITIAL VACUUM OF ABOUT -180 MM.

| Tube No. ....           | Cultures |          |       |       | Water Control | Uninoculated    |               |
|-------------------------|----------|----------|-------|-------|---------------|-----------------|---------------|
|                         | 1        | 2        | 3     | 4     | 5             | Initial Control | Final Control |
| Hrs. ....               | Mm.      | Mm.      | Mm.   | Mm.   | Mm.           | Mm.             | Mm.           |
| 0.....                  | 0        | 0        | 0     | 0     | 0             | 0               | 0             |
| 0.....                  | -183     | -179     | -189  | -187  | -203          | -174            | -176          |
| 24.....                 | 180      | 184      | 190   | 190   | 203           | .....           | 176           |
| 36.....                 | 180      | 183      | 190   | 201   | 202           | .....           | 176           |
| 48.....                 | 183      | 195      | 190   | 209   | 207           | .....           | 176           |
| 72.....                 | -203*    | 213      | 211   | 226   | 215           | .....           | 176           |
| 96.....                 | .....    | 222      | 227   | 228   | 215           | .....           | 176           |
| 114.....                | .....    | -225*    | -229† | 230   | -215          | .....           | 176           |
| 141.....                | .....    | .....    | ..... | -233† | .....         | .....           | -180          |
| Analyses at end of..... | 72 Hrs.  | 114 Hrs. | ..... | ..... | 114 Hrs.      | 0 Hrs.          | 141 Hrs.      |
| CO <sub>2</sub> .....   | 0.20     | 0.00     | ..... | ..... | 6.49          | 0.00            | 0.00          |
| O <sub>2</sub> .....    | 4.41     | 0.76     | ..... | ..... | 0.00          | 9.45            | 8.42          |
| Total.....              | 4.61     | 0.76     | ..... | ..... | 6.49          | 9.45            | 8.42          |

The manometers after evacuation were placed in the hot-room, the barometer reading 729 mm., and temperature of 30 C.

\* The organisms were well formed and active.

† It was impossible to withdraw a sample.

were placed vertically on the sides of the plates, thus holding them together. The remaining 2 inoculated plates were inverted, in like manner, over Petri dishes containing 10 c.c. of water. Two of the plates with alkali and one with water were placed in each of 2 anaerobic jars, which were then sealed in the usual manner. The jars were then connected to a vacuumeter and a tension of -400 mm. was produced in order to favor the liberation of the CO<sub>2</sub>, and, at the same time, to reduce the amount of the dissolved gas. The jars were then placed at 30 C., and incubated for 6 days.

Later, on opening the jars for examination, the water plates were found to be covered with a rich continuous surface growth. The medium was dark,

but the germs were very well formed and actively motile. A striking contrast was presented by the KOH plates; each of them had but 4 colonies, from which thin streamers radiated for a distance of 1 cm. These colonies contained actively motile germs. The medium over the KOH was bright red, unlike that in the plates grown over  $H_2O$ .

This experiment, while not completely successful in demonstrating that no growth could take place in the absence of  $CO_2$ , nevertheless appeared to be somewhat conclusive. But the factor of surface desiccation, due to the action of alkali, must not be overlooked. The great majority of organisms inoculated on the surface of the plates were clearly unable to grow under these experimental conditions. A few organisms, however, seemingly penetrated the medium, and thus securing protection, they were able to multiply although the  $CO_2$  was completely removed from the air which was in contact with the surface of the medium. Small clumps or masses of the inoculum could exert a similar protective action.

*Tr. lewisi* in Petri Dishes over 5% KOH; Exper. 20 B.—The conditions for this experiment were the same as in the preceding one, except that *Tr. lewisi* was used. On opening the jars, all 6 plates were found to be covered with a continuous luxuriant growth of active motile germs. The medium over the water was dark, while that over the KOH was bright red.

The results of this experiment gave no support to the view that  $CO_2$  was necessary for growth, since perfect cultures were obtained in the apparent absence of  $CO_2$ . With the expectation of securing a more definite outcome, the method of procedure was modified, for the next 2 experiments, by substituting a 10% KOH solution for that previously used.

*L. tropica* in Petri Dishes Over 10% KOH; Exper. 20 C.—Six tubes were prepared as in exper. 20 A, the total volume of medium in each tube, however, being 15 c c., to lessen the desiccation arising by the use of a more concentrated reagent. The medium was poured into Petri dishes, which were allowed to stand 12 hours at 34 C. The inoculated plates were then inverted over like dishes containing 10% KOH, and 2 water controls were made as before. The plates were now placed in anaerobic jars, which were evacuated to —400 mm. They were then incubated at 30 C. for 6 days. When examined, the water controls were found to be covered with a luxuriant growth of active germs. The KOH plates, on the other hand, were dotted with small round isolated colonies, about 30 per plate. Examination of these colonies showed perfect organisms. Seemingly little desiccation of the medium was observed, but the plates were not weighed at the start and again at the end of the experi-

ment to determine the exact loss. The results in this experiment are confirmatory of those obtained in exper. 20 A, in which the same organism was used.

*Tr. lewisi* in Petri Dishes Over 10% KOH; Exper. 20 D.—Six plates of medium were prepared as in the previous experiment, *Tr. lewisi* being used as the inoculum. The plates were placed over 10% KOH and H<sub>2</sub>O as before. They were then placed in anaerobic jars, evacuated to —400 mm. and incubated 8 days. On examination, the KOH plates showed no indications of growth, while the water plates were covered with a rich surface growth. This experiment was apparently successful in demonstrating that no growth could take place when the CO<sub>2</sub> was completely removed. Unfortunately, the extent of desiccation was not determined.

In the hope of obtaining more consistent results than those given by the preceding experiments, it was decided to make similar tests substituting serum agar for the medium which had been used. The results of these tests are given in the next 2 experiments. It will be seen that, as regards the need of CO<sub>2</sub>, the outcome was even less satisfactory than in any of the preceding tests.

*L. tropica* on Serum Agar, Over 10% KOH; Exper. 20 E.—To each of 3 tubes containing 10 c.c. of agar, 5 c.c. of rabbit blood serum were added, and the mixture poured into Petri dishes. After standing for 12 hours at 34 C., the plates were inoculated with 2 drops of a culture, and the inoculum was spread over the surface as before. Two of the plates were then inverted over like dishes containing 10 c.c. of 10% KOH. The other plate was placed over water. The plates were now placed in an anaerobic jar and incubated for 6 days. On examination, a rich growth was present on all 3 plates.

*Tr. lewisi* on Serum Agar, Over 10% KOH; Exper. 20 F.—This experiment was prepared in the same way as the previous one, using *Tr. lewisi* as the inoculum. Subsequent examination at the end of 6 days likewise showed rich surface growths on all the plates.

The result of the last 2 experiments in which serum was used instead of whole blood was difficult to explain. The amount of serum was somewhat higher than that present in the 7.5 c.c. of blood used in the preceding tests. It is possible that this represents a higher concentration of carbonates and phosphates, which may have some bearing on the results. On the other hand, it is more likely that the serum agar, by reason of its greater softness, more readily withstood the desiccating action of the alkali, and thus promoted the growth of the organisms.

The 6 foregoing plate experiments may briefly be summarized by stating that in 3 trials with *Tr. lewisi*, one resulted in no growth. In the corresponding 3 tests with *L. tropica*, 2 showed marked inhibition. On the other hand, the 3 trials with *h*-tubes (exper. 18-20) gave



growths although alkali was present in the side-arms. In these, however, minimal amounts of  $\text{CO}_2$  were present during the active stage of multiplication because the absorption of this gas was relatively slower than its production.

In the plate experiments, the conditions for the rapid absorption of  $\text{CO}_2$  were good, but the results were somewhat masked by the desiccation of the medium. It must be evident that an organism cannot possibly grow on a dry surface. The immediate effect of the presence

TABLE 21  
MANOMETRIC READINGS OF CULTURES OF *TR. LEWISI* AND *L. TROPICA* GROWN IN 100%  $\text{H}_2$

|                         | Tr. Lewisii |          |      | L. tropica |          |      | Uninoculated    |               |
|-------------------------|-------------|----------|------|------------|----------|------|-----------------|---------------|
|                         |             |          |      |            |          |      | Initial Control | Final Control |
| Tube No. ....           | 1           | 2        | 3    | 4          | 5        | 6    | 7               | 8             |
| Hrs.                    | Mm.         | Mm.      | Mm.  | Mm.        | Mm.      | Mm.  | Mm.             | Mm.           |
| 0.....                  | 0           | 0        | 0    | 0          | 0        | 0    | 0               | 0             |
| 24.....                 | +1          | 0        | +1   | 0          | +2       | 0    | ....            | 0             |
| 48.....                 | +1          | 0        | 0    | -1         | +2       | 0    | ....            | +1            |
| 72.....                 | +2          | 0        | 0    | 0          | 0        | -1   | ....            | +1            |
| 96.....                 | +1          | -1       | 0    | +1         | 0        | -1   | ....            | -1            |
| 120.....                | +1          | -1       | 0    | 0          | 0        | 0    | ....            | -1            |
| 144.....                | 0           | -1       | -1   | 0          | 0        | -1   | ....            | -1            |
| 168.....                | 0           | -1       | -1   | 0          | +1       | -1   | ....            | -1            |
| 192.....                | 0           | -2       | -1   | -1         | 0        | -1   | ....            | -2            |
| 216.....                | -1          | -2       | 0    | -1         | 0        | -1   | ....            | -2            |
| 240.....                | -1          | -2       | +1   | -1         | +1       | -1   | ....            | -2            |
| 264.....                | -2          | -3       | 0    | -2         | -1       | -2   | ....            | -3            |
| 288.....                | -2          | -3       | -1   | -2         | -1       | -2   | ....            | -3            |
| 336.....                | -2†         | -2       | -1†  | -2†        | -3       | -2†  | ....            | -3            |
| Analyses at end of..... | ....        | 336 Hrs. | .... | ....       | 336 Hrs. | .... | 0 Hrs.          | 336 Hrs.      |
| $\text{CO}_2$ .....     | ....        | 0.16     | .... | ....       | 0.19     | .... | 0.05            | 0.15          |
| $\text{O}_2$ .....      | ....        | 0.03     | .... | ....       | 0.03     | .... | 0.00            | 0.00          |
| Total.....              | ....        | 0.19     | .... | ....       | 0.22     | .... | 0.05            | 0.15          |

The manometers were equilibrated at a barometric pressure of 742 mm. and a temperature of 30° C.

† Not analyzed.

of alkali is to remove all of the moisture on the surface of the medium, and hence inhibition of growth must necessarily take place. It should be added that results similar to those in these experiments were obtained with *B. tuberculosis* (Part II).

#### TR. LEWISI AND L. TROPICA IN THE ABSENCE OF $\text{O}_2$

In all of the experiments which have hitherto been made, these organisms were grown under aerobic conditions. No actual tests had been made to determine whether they were capable of growing under anaerobic conditions. Accordingly, the following experiments with

H<sub>2</sub>, N<sub>2</sub> and CO<sub>2</sub> were planned to determine whether they could be grown in the total absence of O<sub>2</sub>. The results obtained with CO<sub>2</sub> have already been presented under exper. 16.

*Tr. lewisi* and *L. tropica* in 100% H<sub>2</sub>; *Exper. 21*.—For this experiment, *h*-tubes containing blood agar were used. Of these, 3 were inoculated with *Tr. lewisi* and 3 with *L. tropica*, and 2 served as uninoculated controls. These were then attached to manometers and filled with H<sub>2</sub>, then placed in the hot-room and equilibrated as usual.

The results of this experiment are given in table 21. It will be seen that the manometric readings were practically nil. Any growth under these

TABLE 22  
MANOMETRIC READINGS OF CULTURES OF *TR. LEWISI* AND *L. TROPICA* GROWN IN 100% N<sub>2</sub>

| Tube No. ....           | Tr. Lewis |       |          | L. tropica |          |       | Uninoculated    |               |
|-------------------------|-----------|-------|----------|------------|----------|-------|-----------------|---------------|
|                         |           |       |          |            |          |       | Initial Control | Final Control |
| Hrs.                    | 1         | 2     | 3        | 4          | 5        | 6     | 7               | 8             |
| 0.....                  | Mm. 0     | Mm. 0 | Mm. 0    | Mm. 0      | Mm. 0    | Mm. 0 | Mm. 0           | Mm. 0         |
| 12.....                 | 0         | +1    | 0        | +1         | 0        | 0     | ....            | 0             |
| 36.....                 | 0         | +2    | 0        | +1         | 0        | 0     | ....            | 0             |
| 60.....                 | 0         | +1    | 0        | 0          | -1       | -1    | ....            | 0             |
| 84.....                 | +1        | 0     | +1       | 0          | -1       | -1    | ....            | -1            |
| 108.....                | +2        | 0     | +1       | 0          | -1       | 0     | ....            | 0             |
| 132.....                | +2        | 0     | 0        | +1         | -1       | -1    | ....            | 0             |
| 156.....                | +3        | 0     | +1       | 0          | 0        | +1    | ....            | -2            |
| 180.....                | +3        | +1    | 0        | 0          | -1       | 0     | ....            | -3            |
| 204.....                | +2        | 0     | -1       | -1         | -1       | 0     | ....            | -3            |
| 228.....                | +1        | -1    | -2       | -2         | -1       | -1    | ....            | -3            |
| 252.....                | +1        | -1    | -2       | -1         | -1       | -1    | ....            | -3            |
| 276.....                | 0†        | -1†   | -2       | -1†        | -2       | -2†   | ....            | -3            |
| Analyses at end of..... | ....      | ....  | 324 Hrs. | ....       | 324 Hrs. | ....  | 0 Hrs.          | 324 Hrs.      |
| CO <sub>2</sub> .....   | ....      | ....  | 0.18     | ....       | 0.16     | ....  | 0.01            | 0.11          |
| O <sub>2</sub> .....    | ....      | ....  | 0.00     | ....       | 0.00     | ....  | 0.17            | 0.14          |
| Total.....              | ....      | ....  | 0.18     | ....       | 0.16     | ....  | 0.18            | 0.25          |

The manometers were equilibrated at a barometric pressure of 740 mm. and a temperature of 30 C.

† Not analyzed.

conditions would be indicated by a positive reading, which should increase with the duration of the test. The experiment extended over 14 days. No growth was visible macroscopically, and the manometric readings confirmed its absence. The analyses of 2 of the inoculated tubes showed essentially the same composition as that of the 2 controls.

At the close of the experiment the tubes were disconnected and examined; they showed few motile forms. They were then set aside in the incubator, and gave a very rich growth, showing that H<sub>2</sub> had no toxic effect but that the organisms were merely inhibited by the lack of O<sub>2</sub>.

*Tr. lewisi* and *L. tropica* in 100% N<sub>2</sub>; *Exper. 22*.—This experiment was the same as the preceding one except for the substitution of N<sub>2</sub>. As seen in table 22, the results were the same, the manometers indicating no growth.

On disconnecting, a few motile forms were found in each of the tubes. These, on further incubation in air, developed very rich growths, showing, as in the experiment with  $H_2$ , that  $N_2$  itself was not toxic. The absence of growth was therefore wholly due to the lack of  $O_2$ .

TR. LEWISI AND L. TROPICA ON SERUM AGAR

*Tr. brucei* was first cultivated by Novy and MacNeal in 1904. The medium was the same as that used in this work. In 50 attempts, Novy and MacNeal obtained only 4 positive results. Smedley<sup>18</sup> found that 3 out of 10 attempts were positive. Behrens,<sup>9</sup> because of the inconsistent results, attempted to improve the medium. His best results were obtained by the employment of a dialyzed meat extract, made as follows:

One hundred and twenty-five gm. chopped beef, and 250 c.c. of water were allowed to digest over night in the cold. The mixture was then strained and the extract was boiled and filtered. The filtrate was then dialyzed for 24 hours in a large collodium sac against running distilled water. The dialyzed contents of the sac were then diluted to 1 liter with distilled water, and 2% peptone, 0.5% NaCl, 0.01%  $CaCl_2$ , 10 c.c. of a normal solution of  $Na_2CO_3$ , and 2% agar were added. To this nutrient agar, rabbit blood serum was added in a ratio of 2 to 1. On inoculation, he found that this serum agar gave 100% of successful cultures. This medium was used in attempts to cultivate the trypanosomes of sleeping sickness, caderas, surra and dourine, but unsuccessfully.

It was desirable to ascertain the effect, if any, on the gas exchange, of the substitution of serum for whole blood. With this object in view, experiments were made in which serum agar was used in place of blood agar, as had hitherto been the case.

To obtain the serum, the rabbit was bled from the carotid artery into the sterile pipet. Instead of defibrinating, as usual, the pipets were now inclined so as to give, on coagulation, a maximal surface. After the blood had clotted around the defibrinating rod, the pipets were set aside in a vertical position for 24 hours at 10 C. The clear serum, thus obtained, was drawn up into sterile Pasteur bulb pipets, and added to the melted agar in *h*-tubes in the proportion of 1:1, the agar having been previously cooled to 50 C.

*Exper. 23.*—The *h*-tubes, prepared as indicated above, were slanted for 12 hours. They were inoculated with the 2 organisms, attached to manometers, placed in the hot-room, and then finally equilibrated.

The results of this experiment are given in table 23. As regards the manometric readings, it will be seen that the 2 organisms developed the same maximal negative pressure of -45 mm. In the case of *Tr. lewisi*, this point was reached in about twice the time as that with *L. tropica*, which was in accord with the well-known fact that the former multiplied more slowly. The manometric reading of the final control, tube 5, remained zero throughout

<sup>18</sup> Jour. Hyg., 1905, 5, p. 38.

TABLE 23  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF TR. LEWISI AND L. TROPICA GROWN  
ON RABBIT SERUM AGAR

| Tube No. ....           | L. tropica |         | Tr. Lewis! |          | Uninoculated  |                 |
|-------------------------|------------|---------|------------|----------|---------------|-----------------|
|                         | 1          | 2       | 3          | 4        | Final Control | Initial Control |
| Hrs.                    | Min.       | Min.    | Min.       | Min.     | Min.          | Min.            |
| 0.....                  | 0          | 0       | 0          | 0        | 0             | 0               |
| 7.....                  | 0          | 0       | 0          | 0        | 0             | —               |
| 19.....                 | —5         | —6      | 0          | 0        | 0             | ....            |
| 24.....                 | 10         | 12      | 0          | 0        | 0             | ....            |
| 43.....                 | 29         | 31      | —2         | —2       | 0             | ....            |
| 48.....                 | 30         | 35      | 2          | 2        | 0             | ....            |
| 67.....                 | 41         | 45      | 6          | 6        | 0             | ....            |
| 82.....                 | 43         | 46      | 10         | 13       | 0             | ....            |
| 96.....                 | —45*       | —46*    | 14         | 19       | 0             | ....            |
| 114.....                | ....       | ....    | 23         | 30       | 0             | ....            |
| 138.....                | ....       | ....    | 30         | 40       | 0             | ....            |
| 162.....                | ....       | ....    | 36         | 44       | 0             | ....            |
| 186.....                | ....       | ....    | 42         | 45       | 0             | ....            |
| 210.....                | ....       | ....    | —45*       | —45*     | 0             | ....            |
| Analyses at end of..... | 96 Hrs.    | 96 Hrs. | 210 Hrs.   | 210 Hrs. | 210 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 14.56      | 14.69   | 14.46      | 14.56    | 0.57          | 0.20            |
| O <sub>2</sub> .....    | 0.03       | 0.18    | 0.20       | 0.23     | 20.08         | 20.78           |
| Total.....              | 14.59      | 14.87   | 14.66      | 14.79    | 20.65         | 20.98           |

The manometers were equilibrated at a barometric reading of 747 mm., a temperature of 31 C.

\* The cultures were very rich with motile well-formed germs.

TABLE 23a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 23

| Tube No. ....                            | L. tropica |        | Tr. Lewis! |       |
|------------------------------------------|------------|--------|------------|-------|
|                                          | 1          | 2      | 3          | 4     |
| Analyses CO <sub>2</sub> .....           | 14.56      | 14.69  | 14.46      | 14.56 |
| O <sub>2</sub> .....                     | 0.03       | 0.18   | 0.20       | 0.23  |
| N <sub>2</sub> .....                     | 14.59      | 14.97  | 14.66      | 14.79 |
| Total.....                               | 85.41      | 85.13  | 85.34      | 85.21 |
| Corrected analyses CO <sub>2</sub> ..... | 13.47      | 13.60  | 13.32      | 13.50 |
| O <sub>2</sub> .....                     | 0.03       | 0.16   | 0.18       | 0.21  |
| N <sub>2</sub> .....                     | 13.50      | 13.76  | 13.50      | 13.71 |
| Total.....                               | 79.02      | 79.02  | 79.02      | 79.02 |
| Real gain CO <sub>2</sub> .....          | 13.27      | 13.40  | 13.12      | 13.30 |
| Real loss O <sub>2</sub> .....           | 20.75      | 20.62  | 20.60      | 20.57 |
| Respiratory quotient.....                | 0.639      | 0.649  | 0.637      | 0.645 |
| Calc. man. reading.....                  | —53.4      | —51.4  | —53.4      | —51.9 |
| Corr. obs. man. readings.....            | —45.9      | —46.87 | —46.26     | —46.6 |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric readings = real loss × (B — aqueous tension).



TABLE 24  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF TR. LEWISI AND L. TROPICA GROWN  
ON SERUM DIALYZED AGAR

| Tube No. ....           | L. tropica |          | Tr. Lewisl |          | Uninoculated  |                 |
|-------------------------|------------|----------|------------|----------|---------------|-----------------|
|                         | 1          | 2        | 3          | 4        | Final Control | Initial Control |
| Hrs.                    | Mm.        | Mm.      | Mm.        | Mm.      | Mm.           | Mm.             |
| 0.....                  | 0          | 0        | 0          | 0        | 0             | 0               |
| 60.....                 | -12        | -2       | -2         | 0        | 0             | ....            |
| 72.....                 | 16         | 4        | 4          | 4        | 0             | ....            |
| 92.....                 | 24         | 11       | 8          | 6        | 0             | ....            |
| 116.....                | 30         | 18       | 16         | 18       | 0             | ....            |
| 140.....                | 34         | 25       | 24         | 27       | 0             | ....            |
| 164.....                | 38         | 31       | 32         | 36       | 0             | ....            |
| 176.....                | 39         | 33       | 36         | 40       | 0             | ....            |
| 188.....                | 41         | 37       | 38         | -45†     | 0             | ....            |
| 212.....                | -41*       | 40       | -45†       | ....     | 0             | ....            |
| 224.....                | ....       | 45       | ....       | ....     | 0             | ....            |
| 260.....                | ....       | -45*     | ....       | ....     | 0             | ....            |
| Analyses at end of..... | 212 Hrs.   | 260 Hrs. | 212 Hrs.   | 188 Hrs. | 260 Hrs.      | 0 Hrs.          |
| CO <sub>2</sub> .....   | 15.09      | 13.46    | 14.73      | 14.41    | 0.72          | 0.19            |
| O <sub>2</sub> .....    | 0.41       | 1.12     | 0.31       | 0.38     | 20.26         | 20.74           |
| Total.....              | 15.50      | 14.58    | 15.04      | 14.79    | 20.98         | 20.93           |

The manometers were equilibrated at a barometric reading of 750 mm., temperature 31 C.

\* The organisms were completely granulated.

† The organisms were well formed and active.

TABLE 24a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 24

| Tube No. ....                            | L. tropica |        | Tr. Lewisl |        |
|------------------------------------------|------------|--------|------------|--------|
|                                          | 1          | 2      | 3          | 4      |
| Analyses CO <sub>2</sub> .....           | 15.09      | 13.46  | 14.73      | 14.41  |
| O <sub>2</sub> .....                     | 0.41       | 1.12   | 0.31       | 0.38   |
| N <sub>2</sub> .....                     | 15.50      | 14.58  | 15.04      | 14.79  |
| Total.....                               | 84.50      | 85.42  | 84.96      | 85.21  |
| Corrected analyses CO <sub>2</sub> ..... | 100.00     | 100.00 | 100.00     | 100.00 |
| O <sub>2</sub> .....                     | 14.11      | 12.46  | 13.70      | 13.37  |
| N <sub>2</sub> .....                     | 0.38       | 1.08   | 0.28       | 0.35   |
| Total.....                               | 14.49      | 13.49  | 13.98      | 13.72  |
| Real gain CO <sub>2</sub> .....          | 79.07      | 79.07  | 79.07      | 79.07  |
| Real loss O <sub>2</sub> .....           | 98.56      | 92.56  | 98.05      | 92.79  |
| Respiratory quotient.....                | 13.92      | 12.27  | 13.51      | 13.18  |
| Calc. man. readings.....                 | 20.36      | 19.71  | 20.46      | 20.39  |
| Corr. obs. man. readings.....            | 0.683      | 0.623  | 0.660      | 0.645  |
|                                          | -46.12     | -53.3  | -49.78     | -51.6  |
|                                          | -41.54     | -46.08 | -45.76     | -45.72 |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric readings = real loss × (B - aqueous tension).

the experiment. On reference to tables 1 and 3, in which blood agar was used, it will be seen that the corresponding controls gradually developed a slight negative pressure finally reaching  $-8$  mm. This would indicate that the whole blood, because of its cellular elements or because of the method of defibrination, may carry on some respiratory changes which are precluded in the serum agar.

The analyses given in the table are particularly interesting. They revealed the fact that practically all of the  $O_2$  was gone, and that about 14.5% of  $CO_2$  was present. The rate of respiration in this experiment was faster than in tables 1 and 3, but whether this was due to a softer medium or to a larger inoculum, it is not possible to say.

The analytical results obtained in this experiment were recalculated to the nitrogen basis for the purpose of obtaining the real respiratory quotient. It will be seen on reference to table 23a that the quotient for *L. tropica* was found to be 0.639 and 0.649, while that for *Tr. lewisi* gave similar values, namely, 0.637 and 0.645. It should be pointed out, however, that this real quotient is uncorrected for the  $CO_2$  which was dissolved in the medium. More exact determinations of the quotients will be given later.

Microscopically, the germs were actively motile and well shaped. It follows that serum agar was in every respect as good a culture medium as, if not better than, blood agar. No difference, however, was observable in the gas exchange.

*Exper. 24.*—In the preceding experiment, the serum was added to agar which was prepared in the usual way. As pointed out, however, Behrens<sup>9</sup> obtained unusually good results in the cultivation of *Tr. brucei* by employing a dialyzed meat extract. To determine whether this had any advantage over the ordinary agar medium the following experiment was made in which his technic was fully observed. In all other respects, this experiment duplicated the previous one.

The results were practically the same, and from table 24 and 24a it may be concluded that this medium has no advantage over the preceding. The gas exchange was in no wise different, as indicated by the manometric readings of  $-41$  to  $-45$  mm.

The average of the real respiratory quotients of each organism was slightly higher than the corresponding values given in table 23a. Thus, the average value for *L. tropica* was 0.653, as against 0.644 in the former experiment; that for *Tr. lewisi* was 0.652 as contrasted with 0.642.

#### TR. LEWISI AND L. TROPICA ON GLUCOSE MEDIUMS

A considerable literature exists relative to the sparing action of utilizable carbohydrate as regards the proteins in cultures of bacteria. It may be sufficient to mention that Kendall<sup>19</sup> and his co-workers have

<sup>19</sup> *Physiol. Rev.*, 1923, 3, p. 438.

made extensive determinations of the amount of ammonia which is produced in various cultures in the presence or absence of carbohydrate.

In all of the previous experiments in which defibrinated blood or serum was used there was obviously a small amount of carbohydrate present, but probably not to an extent to materially influence the gas exchange. It could be expected that the addition of glucose to such mediums would produce an appreciable difference in the ratio of  $\text{CO}_2$  made and  $\text{O}_2$  consumed; in short, in the respiratory quotient.

The experiments which are to be described were intended to bring out the effect of the addition of glucose to the mediums.

The ordinary glucose mediums are not favorable for the growth of these organisms. They will survive for a few days when planted on plain glucose agar, or in glucose broth, but very little multiplication, if any, takes place. In the experiments which follow, 4% of glucose was added to the agar, and the reaction was readjusted to  $\text{P}_\text{H}$  7.4. After the addition of defibrinated rabbit blood, or serum, the final concentration of glucose became 2%.

*Tr. lewisi* and *L. tropica* on 2% glucose blood agar; *Exper. 25*.—Five *h*-tubes were prepared with this medium. Two were inoculated with *Tr. lewisi* and 2 with *L. tropica*, while the 5th tube was used for an initial control. The tubes were then attached to manometers, placed in the hot-room, and, after 2 hours, equilibrated.

The manometric readings and analyses are given in table 25. Indications of growth were observed at the end of 29 hours by the appearance of dark areas, which rapidly increased in size. At the same time, colonies or masses of organisms became recognizable.

On examination of table 25, a striking fact will be noted, namely, that the maximal negative pressures were only about one-half of those observed in experiments with the usual mediums. The explanation of this behavior was supplied by the analyses. These showed the  $\text{O}_2$  to be practically exhausted and replaced by about 18% of  $\text{CO}_2$ . This high percentage meant that the real loss was less than in previous experiments, and hence the observed manometric readings were necessarily lower.

A recalculation of the analytical values to the nitrogen basis brings out clearly the effect due to the presence of glucose. It will be seen from table 25*a*, for example, that the real gain in  $\text{CO}_2$  was 16.5-17.5%, whereas that in table 24 was less than 13.5. This increase in  $\text{CO}_2$  at once was indicative of a higher respiratory quotient and, as seen from the table, the real quotients were 0.8 or higher, whereas in table 24*a* they were 0.6 or slightly more. Here, as in previous experiments, the respiratory quotients were lower than they really should be, for the reason that more or less  $\text{CO}_2$  was dissolved by the medium. Had

TABLE 25  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *TR. LEWISI* AND *L. TROPICA* GROWN  
ON 2% GLUCOSE BLOOD AGAR

| Tube No. ....           | <i>Tr. Lewisii</i> |          | <i>L. tropica</i> |          | Uninoculated    |
|-------------------------|--------------------|----------|-------------------|----------|-----------------|
|                         | 1                  | 2        | 3                 | 4        | Initial Control |
| Hrs. ....               | Mm.                | Mm.      | Mm.               | Mm.      | Mm.             |
| 0.....                  | 0                  | 0        | 0                 | 0        | 0               |
| 29.....                 | -5                 | 0        | 0*                | 0*       | ....            |
| 63.....                 | 2                  | 0        | -2                | 0        | ....            |
| 75.....                 | 3                  | 0        | 3                 | 0        | ....            |
| 87.....                 | 4                  | 0        | 7                 | 0        | ....            |
| 99.....                 | 7                  | 0        | 9                 | -3       | ....            |
| 111.....                | 13                 | -4       | 15                | 7        | ....            |
| 123.....                | 13                 | 6        | 18                | 9        | ....            |
| 135.....                | 18                 | 10       | 21                | 11       | ....            |
| 147.....                | 18                 | 12       | 23†               | 13†      | ....            |
| 167.....                | 20                 | 17       | 25                | -18‡     | ....            |
| 183.....                | -20‡               | -18‡     | -25‡              | ....     | ....            |
| Analyses at end of..... | 183 Hrs.           | 183 Hrs. | 183 Hrs.          | 167 Hrs. | 0 Hrs.          |
| CO <sub>2</sub> .....   | 18.36              | 18.35    | 17.56             | 17.05    | 0.34            |
| O <sub>2</sub> .....    | 0.13               | 0.15     | 0.05              | 1.56     | 20.43           |
| Total.....              | 18.49              | 18.50    | 17.61             | 18.61    | 20.77           |

The manometers were equilibrated at a barometric reading of 753 mm., a temperature of 31 C.

\* Large dark areas on surface of mediums.

† The surface of the mediums was covered with a heavy mucus-like white growth.

‡ Microscopic examination showed the presence of well-shaped partially granulated active germs.

TABLE 25a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 25

| Tube No. ....                            | <i>Tr. Lewisii</i> |        | <i>L. tropica</i> |        |
|------------------------------------------|--------------------|--------|-------------------|--------|
|                                          | 1                  | 2      | 3                 | 4      |
| Analyses CO <sub>2</sub> .....           | 18.36              | 18.35  | 17.56             | 17.05  |
| O <sub>2</sub> .....                     | 0.13               | 0.15   | 0.05              | 1.56   |
| N <sub>2</sub> .....                     | 18.49              | 18.56  | 17.61             | 18.61  |
|                                          | 81.51              | 81.44  | 82.39             | 81.39  |
| Total.....                               | 100.00             | 100.00 | 100.00            | 100.00 |
| Corrected analyses CO <sub>2</sub> ..... | 17.84              | 17.84  | 16.88             | 16.59  |
| O <sub>2</sub> .....                     | 0.12               | 0.14   | 0.04              | 1.51   |
| N <sub>2</sub> .....                     | 17.96              | 17.98  | 16.92             | 18.10  |
|                                          | 79.23              | 79.23  | 79.23             | 79.23  |
| Total.....                               | 97.19              | 97.21  | 96.15             | 97.33  |
| Real gain CO <sub>2</sub> .....          | 17.50              | 17.50  | 16.54             | 16.25  |
| Real loss O <sub>2</sub> .....           | 20.31              | 20.29  | 20.39             | 18.92  |
| Respiratory quotient.....                | 0.861              | 0.862  | 0.811             | 0.858  |
| Calc. man. reading.....                  | -20.2              | -20.1  | -27.7             | -20.0  |
| Corr. obs. man. reading.....             | -20.2              | -18.5  | -25.27            | -18.49 |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric readings = real loss × (B - aqueous tension).



TABLE 26  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF *L. TROPICA* GROWN ON  
2% GLUCOSE SERUM AGAR

| Tube No. ....           | Cultures |          | Control |
|-------------------------|----------|----------|---------|
|                         | 1        | 2        | 3       |
| Hrs. ....               | Mm.      | Mm.      | Mm.     |
| 0.....                  | 0        | 0        | 0       |
| 22.....                 | -2       | 0        | ....    |
| 34.....                 | 4        | -2       | ....    |
| 46.....                 | 6        | 2        | ....    |
| 58.....                 | 10       | 6        | ....    |
| 70.....                 | 13       | 8        | ....    |
| 82.....                 | 15       | 11       | ....    |
| 94.....                 | 17       | 12       | ....    |
| 106.....                | 20       | 16       | ....    |
| 118.....                | 23       | 19       | ....    |
| 130.....                | 25       | 13       | ....    |
| 142.....                | 27       | 25       | ....    |
| 154.....                | 28       | 27       | ....    |
| 166.....                | 30       | 30       | ....    |
| 178.....                | 30       | 32       | ....    |
| 190.....                | 32       | 35       | ....    |
| 202.....                | 30       | 35       | ....    |
| 214.....                | -32*     | -38*     | ....    |
| Analyses at end of..... | 214 Hrs. | 214 Hrs. | 0 Hrs.  |
| CO <sub>2</sub> .....   | 17.64    | 15.25    | 0.32    |
| O <sub>2</sub> .....    | 0.13     | 1.24     | 20.69   |
| Total.....              | 17.77    | 16.50    | 21.01   |

The manometers were equilibrated, the barometer reading 747 mm., temperature 31 C.

\* The organisms were completely granulated.

TABLE 26a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 26

| Tube No. ....                              | 1      | 2      |
|--------------------------------------------|--------|--------|
| Analyses CO <sub>2</sub> .....             | 17.64  | 15.26  |
| O <sub>2</sub> .....                       | 0.13   | 1.24   |
| N <sub>2</sub> .....                       | 17.77  | 16.50  |
| Total.....                                 | 82.23  | 83.50  |
| Corrected analyses CO <sub>2</sub> .....   | 100.00 | 100.00 |
| O <sub>2</sub> .....                       | 16.94  | 14.43  |
| N <sub>2</sub> .....                       | 0.12   | 1.17   |
| Total.....                                 | 17.06  | 15.60  |
| Real gain CO <sub>2</sub> .....            | 78.99  | 78.99  |
| Real loss O <sub>2</sub> .....             | 96.05  | 94.59  |
| Respiratory quotient.....                  | 16.62  | 14.11  |
| Calculated man. reading.....               | 20.57  | 19.52  |
| Corrected observed manometric reading..... | 0.807  | 0.723  |
|                                            | -28.1  | -38.5  |
|                                            | -32.54 | -38.6  |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric reading = real loss × (B - aqueous tension).

TABLE 27  
MANOMETRIC READINGS AND ANALYSES OF CULTURES OF TR. LEWISI GROWN ON 2% GLUCOSE  
SERUM AGAR AND ON SERUM AGAR

|                        | Glucose Serum Agar |          | Plain Serum Agar |          | Uninoculated    |               |
|------------------------|--------------------|----------|------------------|----------|-----------------|---------------|
|                        |                    |          |                  |          | Initial Control | Final Control |
| Tube No. ....          | 1                  | 2        | 3                | 4        | 5               | 6             |
| Hrs.                   | Mm.                | Mm.      | Mm.              | Mm.      | Mm.             | Mm.           |
| 0.....                 | 0                  | 0        | 0                | 0        | 0               | 0             |
| 65.....                | 0                  | -2       | -1               | -2       | ....            | 0             |
| 89.....                | -7                 | 9        | 6                | 5        | ....            | 0             |
| 102.....               | 7                  | 11       | 7                | 6        | ....            | 0             |
| 125.....               | 14                 | 16       | 15               | 13       | ....            | 0             |
| 137.....               | 16                 | 19       | 20               | 16       | ....            | 0             |
| 149.....               | 19                 | 25       | 23               | 22       | ....            | 0             |
| 173.....               | 20                 | 30       | 31               | 31       | ....            | 0             |
| 198.....               | 28                 | 38       | 33               | 40       | ....            | -2            |
| 209.....               | 30                 | 39       | 36               | 44       | ....            | 2             |
| 258.....               | 25                 | 35       | -43*             | 52       | ....            | 3             |
| 282.....               | -25*               | -35*     | ....             | -52*     | ....            | -3            |
| Analyses at end of.... | 282 Hrs.           | 282 Hrs. | 258 Hrs.         | 282 Hrs. | 0 Hrs.          | 282 Hrs.      |
| CO <sub>2</sub> .....  | 17.00              | 16.80    | 14.41            | 13.72    | 0.08            | 0.70          |
| O <sub>2</sub> .....   | 0.23               | 0.00     | 0.18             | 0.06     | 20.01           | 19.12         |
| Total.....             | 17.23              | 16.80    | 14.59            | 13.78    | 20.09           | 19.82         |

The manometers were equilibrated at a barometric reading of 749 mm., temperature 30 C.

\* The organisms were granulated.

TABLE 27a  
CALCULATED RESPIRATORY QUOTIENTS AND MANOMETRIC READINGS FOR EXPER. 27

| Tube No. ....                            | Glucose Serum Agar |        | Plain Serum Agar |        |
|------------------------------------------|--------------------|--------|------------------|--------|
|                                          | 1                  | 2      | 3                | 4      |
| Analyses CO <sub>2</sub> .....           | 17.00              | 16.80  | 14.41            | 13.72  |
| O <sub>2</sub> .....                     | 0.23               | 0.00   | 0.18             | 0.06   |
| N <sub>2</sub> .....                     | 17.23              | 16.80  | 14.59            | 13.78  |
|                                          | 82.77              | 83.20  | 85.41            | 86.22  |
| Total.....                               | 100.00             | 100.00 | 100.00           | 100.00 |
| Corrected analyses CO <sub>2</sub> ..... | 16.41              | 16.13  | 13.48            | 12.71  |
| O <sub>2</sub> .....                     | 0.22               | 0.00   | 0.16             | 0.05   |
| N <sub>2</sub> .....                     | 16.63              | 16.13  | 13.64            | 12.76  |
|                                          | 79.91              | 79.91  | 79.91            | 79.91  |
|                                          | 96.54              | 96.04  | 93.55            | 92.67  |
| Real gain CO <sub>2</sub> .....          | 16.33              | 16.04  | 13.40            | 12.62  |
| Real loss O <sub>2</sub> .....           | 19.79              | 20.01  | 19.85            | 19.96  |
| Respiratory quotient.....                | 0.825              | 0.798  | 0.675            | 0.633  |
| Calc. man. reading.....                  | -24.74             | -28.3  | -45.4            | -51.71 |
| Corr. obs. man. reading.....             | -25.39             | -35.46 | -44.1            | -52.75 |

Corrected analyses = analytical value × nitrogen factor.

Calculated manometric readings = real loss × (B - aqueous tension).

the dissolved  $\text{CO}_2$  been determined in these tests, the corrected real respiratory quotients, in all probability, would have been found to approximate 1, which is the theoretical value for glucose (tables 30, 31 and 33).

*L. tropica* on 2% Glucose Serum Agar. *Exper. 26.*—In view of the interesting results observed in the previous experiment, it was repeated, but with the substitution of serum for the blood. The data are given in tables 26 and 26a.

It will be seen that the manometric readings were somewhat higher than with blood glucose medium but less than those with plain serum agar. The analyses showed a high  $\text{CO}_2$  content, but the respiratory quotients, though high, did not agree as well as before.

*Tr. lewisi* on 2% Glucose Serum Agar. *Exper. 27.*—This experiment was a continuation of the preceding one except that *Tr. lewisi* was used, and an additional feature was added in that the growth on glucose serum agar was compared with that on plain serum agar, under otherwise identical conditions.

Six tubes were prepared, 4 of which had glucose serum agar and 2 had plain serum agar. Two of the glucose tubes were reserved for controls, and the remaining tubes were inoculated with rich material.

The *h*-tubes were then attached to manometers, placed in the hot-room, and, after the usual period of 2 hours, were equilibrated.

The tubes when examined at the close of the experiment showed a very rich growth, though somewhat granulated. It will be seen from table 27 that the manometric readings of the glucose agar tubes, because of the relatively larger amount of  $\text{CO}_2$ , were appreciably lower than those which were attached to tubes of plain serum agar. It will further be noted that the  $\text{CO}_2$  content in the former was about 3% higher than in the latter, indicating again that the respiratory quotient of the growth on glucose medium would be higher than that on the plain medium.

Reference to table 27a will reveal that the real quotients were about 0.8 for the former and about 0.65 for the latter, which values agreed closely with those given in table 23a. As pointed out heretofore, the real quotients thus obtained are not final, since, in the calculation, only the gaseous  $\text{CO}_2$  is taken into consideration. The true or corrected real quotients are obtained by including all of  $\text{CO}_2$  which is taken up by the rubber and by the medium.

#### CORRECTED REAL RESPIRATORY QUOTIENTS

The fact that man and animals consume oxygen and return  $\text{CO}_2$  was shown by Lavoisier<sup>3</sup> in 1777. He proved animal heat to be due in a large part to the combustion of carbon to  $\text{CO}_2$  in the animal body, and subsequently promulgated the combustion theory of respiration. Dulong,<sup>20</sup> continuing the work, found a difference in the volume of  $\text{CO}_2$  returned per volume of  $\text{O}_2$  consumed in dogs, rabbits and fowls. He suggested that this might be due to a difference in the character of the food. Dulong's suggestion was substantiated by Regnault and Reiset.<sup>13</sup>

readily seen in the experiments which follow. For a full discussion

<sup>20</sup> Ann. d. Chim. et d. Phys., 1841, 3, p. 1.

On the assumption that the oxidation is completed to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , the theoretical respiratory quotient of a carbohydrate is 1.0; of a fat, 0.71 and of a protein, 0.8. If the respiratory quotient is high, carbohydrate is supposedly being oxidized in the body, while if low, fat is undergoing the change. It is clear that in anaerobic respiration  $\text{CO}_2$  is liberated, although no atmospheric  $\text{O}_2$  is consumed.

Moore<sup>21</sup> and his men, working on the respiratory quotients of marine animals, found in all cases a quotient greater than 1. Their observations were based on the determination of dissolved  $\text{O}_2$  in seawater and of the  $\text{CO}_2$  produced. While they accounted for the high quotients by assuming a conversion of carbohydrate to fat with the liberation of  $\text{CO}_2$ , the consumption of  $\text{O}_2$  in the reaction being smaller in amount, it is not clear that they excluded the action of anaerobic micro-organisms which, it is reasonable to believe, were present. In work of this kind, it may be difficult to evaluate correctly the results obtained unless the growth of micro-organisms is excluded.

The calculation of the true real respiratory quotients necessitates exact determination of all of the  $\text{CO}_2$  produced and of the  $\text{O}_2$  consumed. Krogh,<sup>22</sup> in his study of exact respiration in man, pointed out the marked effect of such a factor as vapor tension. Later, Carpenter<sup>23</sup> compared the recent respiratory methods and showed that the great variance in results was due to this and other factors mentioned by Krogh. The respiratory quotients of micro-organisms, as a rule, have been computed from the analysis of the gases over the cultures, but the values thus obtained can at best be considered only approximate. The direct use of the uncorrected analytical data is wrong, since it gives, as pointed out in Part I, the apparent respiratory quotient, which is higher than the real quotient. Another common error is the failure to determine the amount of  $\text{CO}_2$  dissolved in the medium. When the amount of  $\text{CO}_2$  which is held in solution and in combination in the medium is taken into consideration, it will be found that the corrected real respiratory quotient is greater than the real, and may even exceed the apparent quotient.

Another point which must be considered is the period of incubation, which with some organisms may be without appreciable effect. With others, on account of secondary changes in the medium, such as decarboxylation, a longer duration results in an increased  $\text{CO}_2$  production and, hence, in a higher final value. This fact will be

<sup>21</sup> Bio-chem. Jour., 1912, 6, p. 255.

<sup>22</sup> Bio-Chem. Ztschr., 1907, 7, p. 24.

<sup>23</sup> Carnegie Institution, Washington; Publication No. 216.



readily seen in the experiments which follow. For a full discussion of the methods and principles involved in the determination of the true quotient, reference is made to Part I.

In the series of experiments which are to be given, the procedure was essentially the same, the main difference being in the period of incubation and in the culture used.

*Corrected Real Respiratory Quotient of Tr. lewisi on Blood Agar.*—Four tubes (20 x 150 mm.) of blood agar were prepared as usual but with very loose cotton plugs. After having been slanted 12 hours, 2 of the tubes were inoculated, each with 2 drops of a culture of *Tr. lewisi*, and the inoculum was spread with a sterile platinum wire. The 2 uninoculated tubes were used for the control determination of  $\text{CO}_2$  in the medium. The 2 inoculated tubes were placed in an anaerobic jar and, at the same time, 5 drops of distilled water at 80 C. were put on the bottom to hasten the production of aqueous tension. Thereupon the jar was closed, clamped, and attached to a manometer by a rubber stopper (fig. 6, Part I). The manometer and jar were mounted on a common support to relieve all strain from the connection. The apparatus was then placed in the hot-room, with stop-cocks 1 and 3 closed. A positive pressure developed, which was partially relieved by the withdrawal of samples for analysis. After sampling, the manometer was equilibrated. Readings were taken from time to time, and at the end of 28 days the gas content was analyzed. When the analysis was completed, the jar was opened, and the  $\text{CO}_2$  in the culture tubes was determined by the aeration method described in Part I. The analytical data and calculations are given under exper. 4 and in table 28.

It should be noted that the corrected real respiratory quotient is based on gas volumes under standard conditions of temperature and pressure. Assuming protein to be the source of energy, it will be seen that the quotient 0.888 is higher than would be expected. This suggested the possibility that some  $\text{CO}_2$  was made by some other process than respiration. Before discussing the reason for this high value, a similar experiment with *L. tropica* will be given.

*Corrected Real Respiratory Quotient of L. tropica on Blood Agar.*—This experiment was carried on at the same time as the previous one. The data are given in table 29 under exper. 4. The corrected real respiratory quotient (0.907) was even higher than that just given for *Tr. lewisi*. As suggested in connection with that experiment, it is not reasonable to believe that this value actually represents the respiratory process.

The question arose as to what could be the source of  $\text{CO}_2$  other than respiration. If enzymes, whether made by the germs or present in the blood, were acting on the medium, the amount of  $\text{CO}_2$  derived from this source probably should be less with a shorter period of incubation. To test out this point, 6 additional experiments were made, 3 with *Tr. lewisi* and 3 with *L. tropica*. In these, the periods of incubation were varied from 7 to 21 days. To avoid repetition, it may be well to state that in each of these tests the procedure was the same as that given under exper. 28. The data and the results obtained in these 8 experiments, in which the culture medium was blood agar, are given in tables 28 and 29.

*Results with Tr. lewisi.*—The 4 experiments given in table 28 extended over 7, 11, 14 and 28 days, respectively. It will be noted

that manometric readings increased slowly with the duration of the test. Since active multiplication must have ceased after about 10 days, it follows that the subsequent pressure changes were essentially enzymatic in character. Decarboxylation and even deaminization could be expected under these conditions. In either process, bases

TABLE 28  
ANALYSES AND QUOTIENTS OF TR. LEWISI GROWN ON BLOOD AGAR

| Experiment No. ....             | 1       | 2       | 3       | 4       |
|---------------------------------|---------|---------|---------|---------|
| No. tubes.....                  | 2       | 2       | 2       | 2       |
| No. days.....                   | 7       | 11      | 14      | 28      |
| Net air volume.....             | 1617.35 | 2016.35 | 2065    | 2052.12 |
| Barometer.....                  | 748     | 741     | 739     | 757     |
| Temperature, C. ....            | 30      | 31      | 31      | 31      |
| Corr. obs. man. ....            | -1.0    | -2.0    | -4.1    | -5.8    |
| Calc. real man. ....            | -0.8    | -2.1    | -4.4    | -6.5    |
| Analyses                        |         |         |         |         |
| CO <sub>2</sub> .....           | 0.32    | 1.024   | 2.908   | 4.74    |
| O <sub>2</sub> .....            | 20.55   | 19.701  | 17.562  | 15.50   |
| N <sub>2</sub> .....            | 79.13   | 79.275  | 79.54   | 79.76   |
|                                 | 100.00  | 100.000 | 100.000 | 100.00  |
| Corr. analyses*                 |         |         |         |         |
| CO <sub>2</sub> .....           | 0.32    | 1.021   | 2.889   | 4.696   |
| O <sub>2</sub> .....            | 20.52   | 19.642  | 17.441  | 15.358  |
| N <sub>2</sub> .....            | 79.04   | 79.04   | 79.04   | 79.04   |
|                                 | 99.88   | 99.703  | 99.37   | 99.094  |
| C c. at 0 degree, 760 mm.       |         |         |         |         |
| Dissolved CO <sub>2</sub> ..... | 0.16    | 0.25    | 4.23    | 5.10    |
| Gaseous CO <sub>2</sub> .....   | 3.98    | 16.98   | 48.89   | 82.75   |
| Total.....                      | 4.14    | 17.23   | 53.12   | 87.85   |
| O <sub>2</sub> loss.....        | 5.629   | 22.25   | 59.66   | 98.82   |
| Quotients                       | Average |         |         |         |
| Apparent resp. ....             | 0.822   | 0.763   | 0.808   | 0.867   |
| Real resp. ....                 | 0.783   | 0.707   | 0.819   | 0.837   |
| Corr. real resp. ....           | 0.822   | 0.735   | 0.774   | 0.890   |
| CO <sub>2</sub> .....           | 0.050   | 0.040   | 0.015   | 0.061   |

\* The initial gas content was assumed to be that of pure air, viz., CO<sub>2</sub>, 0.03; O<sub>2</sub>, 20.93; and N<sub>2</sub>, 79.04.

would be produced, and hence gradual absorption of CO<sub>2</sub> would take place, resulting in a slow rise in the negative pressure. Attention may be called to the close agreement of the observed and calculated manometric readings.

The analytical values multiplied by the respective nitrogen factor gave the corrected analyses, from which the percentage of real CO<sub>2</sub> gain, and of the real O<sub>2</sub> loss were deduced. The net air volume in the jar was reduced to standard conditions, i.e., dry, at 0 degree and 760 mm., and from this value, the amounts of gaseous CO<sub>2</sub> made, and of O<sub>2</sub> lost were determined in c c.

As indicated in the footnote to the table, it was assumed that the initial gas content was that of pure air. Actual control analyses of the air in the jar at the beginning of each experiment were made, and the results were within the limits of experimental error ( $\pm .03$ ), and for that reason it was believed that the calculations should be based on the standard value.

It is important to note the progressive increase in the amount of  $O_2$  consumed. At the end of 7 days, only 5.6 c.c. were utilized; whereas in 28 days the loss rose to 98.9 c.c. Similarly, the gaseous  $CO_2$  increased from 3.9 to 82.7 c.c.

The increase in the amount of dissolved  $CO_2$ , from 0.1 to 5.1 c.c., is especially to be observed. The latter value is greatly in excess of the amount that could be held in true solution under the partial pressure of  $CO_2$  present. It is indicative, therefore, of the presence of bases resulting, as has been mentioned, either by decarboxylation or deamination or both.

Attention may next be directed to the respiratory quotients. It will be seen at a glance that the corrected real quotients, because of the dissolved  $CO_2$ , were higher than the real quotients and even exceeded the apparent quotients (exper. 3 and 4). On the other hand, the real quotients were lower than the apparent quotients as a necessary consequence of the mode of calculation. Deserving of special mention is the fact that the quotients progressively increased with the duration of the experiment. This was capable of only one interpretation, namely, that additional  $CO_2$  was being made, and that by the process of decarboxylation.

The corrected real quotient rose from 0.735 to 0.888. The question may well be asked as to which of these represents the actual respiratory change of the organism. Without doubt, the truest values are those which are obtained in the experiments in which active multiplication is still taking place. From this point of view, the corrected real quotient for *Tr. lewisi*, when growing on blood agar, should approximate the average of the values obtained on the 7th and 11th days; namely, 0.754. The average of the 4 determinations given in the table was 0.822.

*Results with L. tropica.*—The 4 experiments with this organism extended over 7, 11, 21 and 28 days, respectively, and are summarized in table 29.

It should be pointed out, that since *L. tropica* grows more rapidly than *Tr. lewisi*, the gas changes in the jars were more marked, especially during the first 7-11 days. Thus, the analysis on the 7th day revealed a gain in gaseous  $\text{CO}_2$  of 24.4 c.c., as against 3.9 c.c. made

TABLE 29  
ANALYSES AND QUOTIENTS OF *L. TROPICA* GROWN ON BLOOD AGAR

| Experiment No. ....             | 1       | 2       | 3      | 4       |
|---------------------------------|---------|---------|--------|---------|
| No. tubes.....                  | 2       | 2       | 2      | 2       |
| No. days.....                   | 7       | 11      | 21     | 28      |
| Net air volume.....             | 2020.41 | 2054.12 | 2127   | 2014.41 |
| Barometer.....                  | 748     | 741     | 752    | 746     |
| Temperature, C. ....            | 30      | 31      | 31     | 31      |
| Corr. obs. man. ....            | -2.0    | -3.0    | -3.4   | -4.0    |
| Calc. real man. ....            | -2.4    | -3.7    | -3.9   | -4.7    |
| Analyses                        |         |         |        |         |
| CO <sub>2</sub> .....           | 1.47    | 2.059   | 2.92   | 3.90    |
| O <sub>2</sub> .....            | 19.22   | 18.488  | 17.61  | 16.53   |
| N <sub>2</sub> .....            | 79.31   | 79.453  | 79.47  | 79.57   |
|                                 | 100.00  | 100.000 | 100.00 | 100.00  |
| Corr. analyses*                 |         |         |        |         |
| CO <sub>2</sub> .....           | 1.46    | 2.048   | 2.90   | 3.874   |
| O <sub>2</sub> .....            | 19.15   | 18.400  | 17.51  | 16.419  |
| N <sub>2</sub> .....            | 79.04   | 79.04   | 79.04  | 79.04   |
|                                 | 99.65   | 99.488  | 99.45  | 99.333  |
| C c. at 0 degree, 760 mm.       |         |         |        |         |
| Dissolved CO <sub>2</sub> ..... | 1.09    | 2.215   | 4.23   | 4.23    |
| Gaseous CO <sub>2</sub> .....   | 24.43   | 34.44   | 52.86  | 64.73   |
| Total.....                      | 25.52   | 36.655  | 57.09  | 68.96   |
| O <sub>2</sub> loss.....        | 30.42   | 43.18   | 62.99  | 75.965  |
| Quotients                       |         |         |        |         |
| Apparent resp. ....             | 0.855   | 0.842   | 0.870  | 0.879   |
| Real resp. ....                 | 0.822   | 0.803   | 0.839  | 0.852   |
| Corr. real resp. ....           | 0.875   | 0.839   | 0.848  | 0.907   |
| CO <sub>2</sub> .....           | 0.063   | 0.044   | 0.064  | 0.065   |

\* See footnote to table 28.

by *Tr. lewisi* in the same time. With such active respiratory change, it was to be inferred that protein cleavage would likewise be more evident. Actually, the determination of the dissolved  $\text{CO}_2$  showed the presence of 1.1 c.c. as against 0.16 in the case of *Tr. lewisi*.

It will be seen from table 29 that the quotients increased in value with the duration of the experiment, rising from 0.839 to 0.907, the average being 0.875. These values were clearly higher than would be expected for direct protein combustion. Significant is the fact that the average for the corrected real quotients, on the 7th and 11th day, was 0.843 as against 0.754 of the slower growing *Tr. lewisi*. This marked difference may be due to excess of  $\text{CO}_2$  production by decarboxylation.



Possibly, determinations of the quotient on the 3rd or 5th day might give a lower value which would approximate 0.754, that obtained for *Tr. lewisi*. However, it is well to bear in mind that, in air, the rapidly growing *L. tropica*, unlike *Tr. lewisi*, produces a marked change in the color of the blood agar. This reaction may be expressive of early and intense decarboxylation changes, and, if so, it may be difficult to exclude the latter.

TABLE 30  
ANALYSES AND QUOTIENTS OF *TR. LEWISI* GROWN ON GLUCOSE BLOOD AGAR.

| Experiment No. ....             | 1       | 2       | 3       |
|---------------------------------|---------|---------|---------|
| No. tubes.....                  | 2       | 2       | 2       |
| No. days.....                   | 7       | 14      | 21      |
| Net air volume.....             | 2016.77 | 1782.67 | 2206.8  |
| Barometer.....                  | 754     | 754     | 754     |
| Temperature, C.....             | 31      | 31      | 31      |
| Corr. obs. man. ....            | 0.0     | 0.0     | 0.0     |
| Calc. real man. ....            | -0.6    | -1.1    | -0.5    |
| Analyses                        |         |         |         |
| CO <sub>2</sub> .....           | 1.11    | 2.934   | 1.446   |
| O <sub>2</sub> .....            | 19.79   | 17.907  | 19.427  |
| N <sub>2</sub> .....            | 79.10   | 79.159  | 79.127  |
|                                 | 100.00  | 100.000 | 100.000 |
| Corr. analyses*                 |         |         |         |
| CO <sub>2</sub> .....           | 1.10    | 2.93    | 1.444   |
| O <sub>2</sub> .....            | 19.77   | 17.88   | 19.406  |
| N <sub>2</sub> .....            | 79.04   | 79.04   | 79.04   |
|                                 | 99.91   | 99.85   | 99.890  |
| C c. at 0 degree, 760 mm.       |         |         |         |
| Dissolved CO <sub>2</sub> ..... | 0.23    | 0.10    | 0.00    |
| Gaseous CO <sub>2</sub> .....   | 18.34   | 43.93   | 26.52   |
| Total CO <sub>2</sub> .....     | 18.57   | 44.03   | 26.52   |
| O <sub>2</sub> loss.....        | 18.89   | 46.20   | 28.59   |
| Quotients                       | Average |         |         |
| Apparent resp. ...              | 0.950   | 0.947   | 0.961   |
| Real resp. ....                 | 0.933   | 0.922   | 0.950   |
| Corr. real resp. ...            | 0.938   | 0.934   | 0.953   |
| CO <sub>2</sub> .....           | 0.004   | 0.012   | 0.002   |
|                                 |         |         | .....   |

\* See footnote to table 28.

On the other hand, the energetic respiration of the organism may involve the combustion of substances which *Tr. lewisi* was not able to utilize. While, in general, it is to be expected that for a given medium the quotient will be constant, irrespective of the kind of organism at work, still, in view of the complexity of such a medium, selective combustion is possible and should be borne in mind.

In view of the foregoing results obtained with blood agar, it was desirable to determine the respiratory quotients for these organisms

when grown on the same medium to which 2% of glucose had been added. The method of procedure was otherwise the same as that given in connection with table 28.

*Corrected Real Respiratory Quotient of Tr. lewisi on Glucose Blood Agar.*—The results obtained in 3 experiments with this medium will be found in table 30. They should be carefully compared with those given in table 28. The gas exchange in exper. 3 was less than in the others, due without doubt to a poor inoculation, and hence to a more feeble growth.

A rather striking difference, to which attention should be directed at the outset, is to be seen in the amount of dissolved  $\text{CO}_2$ . After allowing for the  $\text{CO}_2$  present in the uninoculated control tube, the amount of the dissolved  $\text{CO}_2$  was practically negligible, being 0.23, 0.10 and 0.0, respectively. By contrast, the results in table 28 show a progressive increase from 0.16 to 5.10 c.c. Clearly, the presence of glucose appears to prevent the decarboxylation, if not deamination, which is so evident in the tests with plain blood agar. It may be regarded as a new expression of the so-called protein-sparing action of carbohydrates.

At the end of 7 days the  $\text{O}_2$  loss (19.9 c.c.) was much greater than at the corresponding time in cultures grown on blood agar (5.6 c.c.). On the other hand, at the close of 14 days, it was inferior, being 46.2 as against 59.6.

Relatively, the amount of  $\text{CO}_2$  produced on the glucose medium was higher, and this is particularly evident on comparison of the respiratory quotients. On account of the minimal amounts of dissolved  $\text{CO}_2$ , there is much less difference between the values found of the corresponding real and the corrected real quotients than is the case in table 28. Moreover, there is little or no progressive increase in the values of the quotients according to the duration of the experiment, which fact supports the conclusion arrived at above, namely, the absence of decarboxylation.

On the 7th day, the corrected real respiratory quotient was found to be 0.934 as against 0.735 obtained with the blood agar medium. The average for the 3 experiments was 0.938.

It is evident from the value just given that 1, the theoretical quotient for glucose, was not realized. It merely goes to show that *Tr. lewisi* when growing on glucose blood agar does not obtain all of the requisite energy from glucose. If it did, then without doubt the quotient would have been 1, as has been shown to be the case for

B. tuberculosis, in Part II. It is evident, therefore, that in addition to glucose, it also utilizes some protein as a source of energy, and as a result the quotient is depressed. This depression is such as might be expected from the combustion of 3 parts of glucose and 1 part of protein, assuming the quotient of the latter to be 0.73.

TABLE 31  
ANALYSES AND QUOTIENTS OF *L. TROPICA* GROWN ON GLUCOSE BLOOD AGAR

| Experiment No. ....             | 1       | 2       | 3       |
|---------------------------------|---------|---------|---------|
| No. tubes.....                  | 2       | 2       | 2       |
| No. days.....                   | 7       | 14      | 21      |
| Net air volume.....             | 2112.41 | 1591.07 | 2158.35 |
| Barometer.....                  | 754     | 754     | 754     |
| Temperature, C.....             | 31      | 31      | 31      |
| Corr. obs. man. ....            | 0.0     | 0.0     | 0.0     |
| Calc. real man. ....            | -1.58   | -3.16   | -3.58   |
| Analyses                        |         |         |         |
| CO <sub>2</sub> .....           | 2.748   | 7.200   | 6.49    |
| O <sub>2</sub> .....            | 18.042  | 13.411  | 14.09   |
| N <sub>2</sub> .....            | 79.210  | 79.389  | 79.42   |
|                                 | 100.000 | 100.000 | 100.00  |
| Corr. analyses*                 |         |         |         |
| CO <sub>2</sub> .....           | 2.74    | 7.168   | 6.459   |
| O <sub>2</sub> .....            | 18.00   | 13.352  | 14.022  |
| N <sub>2</sub> .....            | 79.04   | 79.04   | 79.04   |
|                                 | 99.78   | 99.560  | 99.521  |
| C c. at 0 degree, 760 mm.       |         |         |         |
| Dissolved CO <sub>2</sub> ..... | 1.23    | 1.73    | 1.83    |
| Gaseous CO <sub>2</sub> .....   | 48.64   | 96.23   | 117.69  |
| Total CO <sub>2</sub> .....     | 49.87   | 97.96   | 119.52  |
| O <sub>2</sub> loss.....        | 52.55   | 102.16  | 126.24  |
| Quotients                       | Average |         |         |
| Apparent resp. ...              | 0.916   | 0.953   | 0.944   |
| Real resp. ....                 | 0.932   | 0.941   | 0.930   |
| Corr. real resp. ...            | 0.951   | 0.959   | 0.946   |
| CO <sub>2</sub> .....           | 0.019   | 0.018   | 0.015   |

\* See footnote to table 28.

*Corrected Real Respiratory Quotient of L. tropica on Glucose Blood Agar.*—A set of 3 experiments, similar to those just given for *Tr. lewisi*, were made with *L. tropica*, and the results obtained are given in table 31.

On comparing this with table 29, several interesting facts will be evident. Thus, the amount of dissolved CO<sub>2</sub> ranged from 1.2 to 1.8, while, for the blood agar medium, it rose from 1.1 to 4.2. It would appear that with the active and rapidly growing *L. tropica*, the carboxylase action was only partly suppressed as compared with the effect seen in the experiments with *Tr. lewisi* (table 30).

The  $O_2$  loss in 7 to 21 days rose from 52.5 to 126.2 as compared with 30.4 to 62.9 on plain blood agar. Hence, in 21 days, the  $O_2$  consumption, per tube, was 63 c.c. for glucose blood agar, and 31.5 c.c. for plain blood agar.

The  $CO_2$  gain, similarly, increased from 49.8 to 119.5, whereas on plain blood agar it went from 25.5 to 57.1. On the 21st day, per tube, the  $CO_2$  gain was approximately 60 and 28.5 c.c., respectively.

The corrected real respiratory quotients obtained in the 3 experiments showed a satisfactory agreement, varying only from 0.946 to 0.959, with an average of 0.951. The average for *Tr. lewisi* on the same medium was 0.938, the difference being due to the relative amounts of dissolved  $CO_2$ .

As in the case of *Tr. lewisi*, the respiratory quotient of 1 was not realized in these tests with *L. tropica*. The presence of glucose did not completely suppress the combustion of proteins, or the action of carboxylase; hence, the quotient of 0.95 instead of 1.0, as was expected.

In arriving at the corrected real respiratory quotients given in the preceding tables, the utmost care was taken to obtain true values for the dissolved  $CO_2$ . It should be pointed out that these determinations were the least accurate, since they involved the removal of the gas from the finely comminuted medium. Possibly, better values could be obtained if the medium could be completely liquefied, but this was impossible with blood mediums. The amount of  $CO_2$  taken up by the blood agar medium was very large, especially so when the incubation was carried on for 14 days or more, as will be seen on reference to tables 28 and 29.

It must be remembered in this connection that, in the foregoing experiments, anaerobic jars of about 2,000 c.c. capacity were used. Consequently, the concentration of the free  $CO_2$  could at no time reach that which was found when *h*-tubes were employed. In the latter, the  $O_2$  had practically disappeared, and about 14% of  $CO_2$  was present. It follows, therefore, that a relatively larger amount of  $CO_2$  should be taken up by the medium in *h*-tubes than was the case in the jar experiments. That this actually occurred will be seen on reference to table 32.

The net air volume, unreduced, in the *h*-tubes was about 100 c.c. It is to be noted that, largely because of the high percentage of  $CO_2$  (13-15%), with one exception, the dissolved  $CO_2$  was in excess of 5 c.c. In jar exper. 2 of table 29, of the same duration, the dissolved  $CO_2$  was 2.2 c.c., while the gaseous  $CO_2$  was 2.05%.



A comparison, in the case of tubes 1 and 2, of the actual volumes of dissolved and of gaseous CO<sub>2</sub> shows that the former was nearly one-half of the latter. The effect of this large amount of dissolved CO<sub>2</sub> on the calculation of the respiratory quotients was marked. Thus, while the real quotients in table 32 were about 0.15 lower than those in table 29, the corrected real quotients were more than 0.1 higher

TABLE 32

L. TROPICA ON BLOOD AGAR, IN *h*-TUBES, AT 31 C.: SHOWING EFFECT OF RUBBER STOPPERS ON CONTENT OF CO<sub>2</sub> AND ON THE QUOTIENT VALUES

| Closed with.....               | Ground Glass Caps |         |        |        |        | Rubber Stoppers |        |        |        |        |
|--------------------------------|-------------------|---------|--------|--------|--------|-----------------|--------|--------|--------|--------|
| Exper. No. ....                | 1                 |         | 2      |        |        | 2a              |        |        |        |        |
| Tube No. ....                  | 1                 | 2       | 3      | 4      | 5c     | 6               | 7      | 8      | 9c     | 10c    |
| Duration, hrs. ....            | 240               | 264     | 240    | 264    | 240    | 240             | 240    | 264    | 12     | 264    |
| Corr. obs. man. ....           | 43                | 44.2    | 44.2   | 46.1   | 0      | 63.6            | 61.9   | 57.4   | 0      | 7      |
| Calc. real man. ....           | 43.2              | 46.6    | 51.2   | 51.6   | .....  | 63.6            | 61.9   | 61.3   | .....  | .....  |
| Analyses                       |                   |         |        |        |        |                 |        |        |        |        |
| CO <sub>2</sub> .....          | 15.447            | 15.456  | 14.86  | 14.80  | 1.16   | 13.24           | 13.46  | 13.54  | 0.286  | 1.25   |
| O <sub>2</sub> .....           | 0.0               | 0.0     | 0.0    | 0.0    | 19.61  | 0.0             | 0.0    | 0.0    | 20.596 | 19.27  |
| N <sub>2</sub> .....           | 84.553            | 84.544  | 85.14  | 85.20  | 79.23  | 86.76           | 86.54  | 86.46  | 79.119 | 79.48  |
|                                | 100.000           | 100.000 | 100.00 | 100.00 | 100.00 | 100.00          | 100.00 | 100.00 | 100.00 | 100.00 |
| Corr. analyses*                |                   |         |        |        |        |                 |        |        |        |        |
| CO <sub>2</sub> .....          | 14.44             | 14.45   | 13.795 | 13.730 | .....  | 12.062          | 12.293 | 12.378 | .....  | .....  |
| O <sub>2</sub> .....           | 0.0               | 0.0     | 0.0    | 0.0    | .....  | 0.0             | 0.0    | 0.0    | .....  | .....  |
| N <sub>2</sub> .....           | 79.04             | 79.04   | 79.04  | 79.04  | .....  | 79.04           | 79.04  | 79.04  | .....  | .....  |
|                                | 93.48             | 93.49   | 92.835 | 92.77  |        | 91.102          | 91.333 | 91.418 |        |        |
| C c. at 0 degree,<br>760 mm.   |                   |         |        |        |        |                 |        |        |        |        |
| Dissolved CO <sub>2</sub> .... | 5.120             | 5.431   | 9.23   | 5.63   | 0.87   | 3.89            | 5.34   | 5.28   | .....  | 0.78   |
| Gaseous CO <sub>2</sub> ....   | 11.73             | 12.176  | 11.5   | 11.37  | .....  | 9.19            | 8.91   | 9.26   | .....  | .....  |
| Total CO <sub>2</sub> ....     | 16.85             | 17.607  | 20.73  | 17.05  | .....  | 13.08           | 14.25  | 14.54  | .....  | .....  |
| O <sub>2</sub> loss.....       | 17.037            | 17.673  | 17.49  | 17.37  | .....  | 15.99           | 15.21  | 15.69  | .....  | .....  |
| Quotients                      |                   |         |        |        |        |                 |        |        |        |        |
| Apparent resp. ..              | 0.736             | 0.737   | 0.709  | 0.705  | .....  | 0.631           | 0.641  | 0.645  | .....  | .....  |
| Real resp. ....                | 0.689             | 0.689   | 0.658  | 0.654  | .....  | 0.575           | 0.586  | 0.590  | .....  | .....  |
| Corr. real resp. ..            | 0.969             | 0.996   | 1.180  | 0.981  | .....  | 0.818           | 0.937  | 0.927  | .....  | .....  |
| CO <sub>2</sub> .....          | 0.436             | 0.446   | 0.800  | 0.499  | .....  | 0.423           | 0.579  | 0.570  | .....  | .....  |

\* See footnote to table 28.

5c, 9c and 10c were uninoculated controls.

The dissolved CO<sub>2</sub> in nos. 1 and 2 represents the value found less 0.387, the amount present in a control tube at start of experiment.

In nos. 3, 4, 6, 7 and 8 the deduction was 0.78, which was the amount present in control 10 at the close of the experiment.

than in the latter table, and actually approximated 1. Incidentally, attention should be called to the high values for the CO<sub>2</sub> quotients, which were much higher than those given in table 29.

Table 32 is primarily intended to bring out the fact that an appreciable loss of CO<sub>2</sub> resulted when rubber stoppered *h*-tubes were used. These were always attached to the manometers by means of no. 3 or 4 rubber stoppers treated with glycerol, and the latter, as was shown

in table 16, will absorb  $\text{CO}_2$ , thus reducing the amount of  $\text{CO}_2$  over the culture. It was desirable to ascertain how great was the loss of  $\text{CO}_2$  due to the rubber stopper. For this purpose, *h*-tubes were provided with ground glass caps, such as are shown in fig. 2*B*, Part I. After inoculation, these tubes (nos. 1-5) were attached to the manometers by means of no. 25 rubber stoppers, thus making glass

TABLE 33  
AVERAGES OF DETERMINATIONS OF RESPIRATORY QUOTIENTS

| Respiratory quotient.....           | L. tropica |            | Tr. Lewisii |            |
|-------------------------------------|------------|------------|-------------|------------|
|                                     | Real       | Corr. Real | Real        | Corr. Real |
| Blood agar                          |            |            |             |            |
| <i>h</i> -tube, rubber stopper..... | 0.648 (19) | .....      | 0.658 (13)  | .....      |
| Glass cap.....                      | 0.672 (4)  | 0.992 (2)  | .....       | .....      |
| Jars.....                           | 0.822 (4)  | 0.875 (4)  | 0.783 (4)   | 0.822 (4)  |
| Glucose blood agar                  |            |            |             |            |
| <i>h</i> -tube rubber stopper.....  | 0.800 (4)  | .....      | 0.838 (4)   | .....      |
| <i>h</i> -tube rubber stopper.....  | 0.858 (5)  | 0.935 (5)  | .....       | .....      |
| Jars.....                           | 0.932 (3)  | 0.951 (3)  | 0.933 (3)   | 0.938 (3)  |

The figures in parentheses indicate the number of tests which were averaged.

TABLE 34  
GAS CHANGES, PER TUBE OF TR. LEWISII AND L. TROPICA GROWN ON BLOOD AGAR AND ON GLUCOSE BLOOD AGAR (TABLES 28-31)

| Medium.....                    | Blood Agar |        |        |        | Glucose Blood Agar |        |        |
|--------------------------------|------------|--------|--------|--------|--------------------|--------|--------|
|                                | 7          | 11     | 14     | 28     | 7                  | 14     | 21     |
| Days.....                      |            |        |        |        |                    |        |        |
| Tr. Lewisii                    |            |        |        |        |                    |        |        |
| At 0 degree and 760 mm.        |            |        |        |        |                    |        |        |
| $\text{CO}_2$ made.....        | 2.07       | 8.61   | 26.51  | 43.92  | 9.28               | 22.01  | 13.26  |
| $\text{O}_2$ lost.....         | 2.81       | 11.12  | 29.83  | 49.41  | 9.94               | 23.01  | 14.30  |
| At 30 degrees and 750 mm.      |            |        |        |        |                    |        |        |
| $\text{CO}_2$ made.....        | 2.43       | 10.11  | 31.14  | 51.53  | 10.90              | 25.85  | 15.57  |
| $\text{O}_2$ lost.....         | 3.30       | 13.06  | 34.92  | 58.03  | 11.67              | 27.02  | 16.79  |
| Air volume.....                | 15.77      | 62.40  | 167.40 | 277.28 | 55.78              | 129.13 | 80.25  |
| Percentage $\text{CO}_2$ ..... | 15.38      | 16.20  | 18.63  | 18.58  | 19.55              | 19.94  | 19.40  |
| Days.....                      | 7          | 11     | 21     | 28     | 7                  | 14     | 21     |
| L. tropica                     |            |        |        |        |                    |        |        |
| At 0 degree and 760 mm.        |            |        |        |        |                    |        |        |
| $\text{CO}_2$ made.....        | 12.76      | 18.32  | 28.54  | 34.48  | 24.93              | 48.98  | 59.76  |
| $\text{O}_2$ lost.....         | 15.21      | 21.59  | 31.49  | 37.93  | 26.27              | 51.06  | 63.12  |
| At 30 degrees and 750 mm.      |            |        |        |        |                    |        |        |
| $\text{CO}_2$ made.....        | 14.99      | 21.52  | 33.52  | 40.50  | 29.28              | 57.53  | 70.19  |
| $\text{O}_2$ lost.....         | 17.86      | 25.36  | 36.98  | 44.55  | 31.18              | 60.00  | 74.14  |
| Air volume.....                | 85.35      | 121.16 | 176.72 | 212.85 | 147.42             | 286.65 | 354.22 |
| Percentage $\text{CO}_2$ ..... | 17.56      | 17.74  | 18.96  | 18.96  | 19.86              | 20.07  | 19.79  |

to glass connections. Tubes 6 to 10 were attached in the usual way, as in all of the previous experiments. Experiments 2 and 2*a* were made at the same time, and the results are, therefore, directly comparable. Experiment 1, it should be added, was made at a different time.

The observed manometric readings of the tubes which were provided with rubber stoppers (nos. 6-8) were about 15 mm. higher

than those with glass caps (nos. 1-4). This in itself indicated a loss of about 2% of  $\text{CO}_2$ . The analyses confirmed this conclusion, since the tubes with rubber stoppers had only about 13%, while the glass-capped tubes had about 15% of  $\text{CO}_2$ . The average real loss as deduced from the corrected analyses for tubes 6-8 was 8.7, while that for tubes 1-4 was 6.3, the difference being due to the actual loss of  $\text{CO}_2$  by solution in the rubber stopper.

The effect of this loss of  $\text{CO}_2$  on the respiratory quotients was considerable. The average for the apparent quotients of the glass-capped tubes was 0.722, as against 0.639 of those with rubber stoppers. Similarly, the real quotients average 0.672 and 0.584, respectively. The corrected real quotients for nos. 1 and 2, because of the relatively large amount of dissolved  $\text{CO}_2$ , averaged 0.993, whereas for the jar experiments in table 29, they averaged 0.875.

Another source of error is the uncertainty of the exact composition of the air which is present in the culture tubes at the start of an experiment. The air is subject to variation in composition, even in a given set of tubes. This error is minimized when jars instead of tubes are used as the respiratory chambers.

The value of the real respiratory quotient may be depressed, as has been shown above, by a loss of  $\text{CO}_2$ . This quotient may also be lowered as the result of direct absorption of some  $\text{O}_2$  by the reducing products of the organism. On the other hand, the production of  $\text{CO}_2$  by carboxylase action would cause an increase in the quotient.

In table 33 are given the averages for the real and corrected real quotients as recorded in all of the previous tables. It will be seen at a glance that the real quotient was lowest when the *h*-tubes were used in the ordinary way. When used with glass caps, the values were appreciably higher but still inferior to those obtained with jars. Again, the corrected real quotients for *L. tropica* were higher than those for *Tr. lewisi*.

It is evident from the table that there is an appreciable error when the *h*-tubes are attached to the manometers in the usual way. The results are only approximate because of the loss of  $\text{CO}_2$ . This loss can be reduced by using either glass-capped *h*-tubes, or glass-stoppered side-arm tubes. Obviously, the quotients obtained with Novy jars are free of this and other errors, incidental to the use of tubes, and are therefore to be accepted as the true values.

*Gas Changes per Tube of Tr. lewisi and L. tropica.*—From the jar experiments given in tables 28-31, it is possible to deduce values which

represent the gas changes brought about by the developing culture within a single tube. The instructive results thus obtained are brought together in table 34.

The table gives, in the first place, the number of c.c. of  $\text{CO}_2$  made and of  $\text{O}_2$  consumed by a culture at the end of the several periods of incubation. These results express the values under standard conditions, i. e., 0 degree and 760 mm., dry.

In order to show the corresponding values, under the conditions of cultivation, the foregoing results were recalculated to 30 C. and 750 mm. The formula for this conversion was given in Part II and for the conditions mentioned it becomes

$$V = V_0 \times 1.17455.$$

Multiplying the gas volumes, under standard conditions, by this factor gave the values for  $\text{CO}_2$  made and  $\text{O}_2$  consumed at 30 C. and 750 mm.

Further, to obtain a definite idea of the volume of air utilized by a culture, the number of c.c. of  $\text{O}_2$  consumed under standard conditions was multiplied by 5.6118. This value was arrived at by multiplying the foregoing factor (1.17455) by 100 and dividing by 20.93, the oxygen content of pure air.

It will be seen from the table that the gas changes due to *L. tropica* on blood agar, on the 7th day, were more than 5 times those of *Tr. lewisi*. On the 11th day, they were twice as large, but after that the difference became less, so that seemingly by the 14th day they became inferior to those of *Tr. lewisi*. It is worth while to point out that about 100 c.c. of air were needed to obtain good rich cultures of either organism.

The gas changes on glucose blood agar on the 7th day were greater than those on the usual medium. With *Tr. lewisi*, the difference was nearly 4-fold, while with *L. tropica* it was only about double.

The table also contains values which represent the percentage of  $\text{CO}_2$  which would be found in the tube provided no  $\text{CO}_2$  was taken up by the medium. These values were based on the corrected real respiratory quotients, as given in tables 28-31, and on the assumption that all of the  $\text{O}_2$  present in the air (20.93) was consumed, the formula being:

$$\text{Percentage of } \text{CO}_2 \text{ produced} = \text{corrected real} \\ \text{quotient} \times \text{percentage of } \text{O}_2 \text{ consumed (20.93)}.$$

It has been shown (e. g., table 32) that the gas in a culture tube, on analysis, contained 13-15% of  $\text{CO}_2$  when all of the  $\text{O}_2$  had been consumed. Obviously, these figures did not represent the total yield,



since a considerable volume of  $\text{CO}_2$  was taken up by the medium. The real percentage of  $\text{CO}_2$  produced, as calculated in table 34, ranged from 15-19% for blood agar. The higher values, in this case, as pointed out heretofore, were probably due to secondary  $\text{CO}_2$  production. The values for the glucose blood agar were more uniform, and corresponded closely to what was to be expected for the respiratory quotient of glucose.

#### SUMMARY

For the first time, a detailed study of the respiration of pure cultures of two well-known protozoa is presented.

The results show that the gas exchange is the same as in the case of bacteria, although in this paper no direct comparison is made. That this is true will be seen on reference to Part II, which deals with the respiration of *B. tuberculosis*.

When grown in tubes which have a capacity of about 100 c.c. of air, *L. tropica* consumed all of the  $\text{O}_2$  in about 144 hours; whereas *Tr. lewisi* required about twice that time. The yield of  $\text{CO}_2$  was about the same with both organisms, amounting to about 15%, uncorrected for the dissolved gas.

When grown in jars which have a capacity of about 2,000 c.c., 2 tubes of *L. tropica*, in 28 days, produced 68.96 c.c. of  $\text{CO}_2$  and consumed 75.96 c.c. of  $\text{O}_2$ . Similarly, 2 tubes of *Tr. lewisi* produced 87.85 c.c. of  $\text{CO}_2$  and consumed 98.82 c.c. of  $\text{O}_2$ . These values are calculated for standard conditions, 0 degree and 760 mm.

The manometer is an excellent index of the growth of these organisms. The readings obtained vary with the  $\text{O}_2$  concentration; thus, when air was present in the culture tube, the maximal negative pressure was about 50 mm. In 50%  $\text{O}_2$ , *L. tropica* gave readings as high as —157 mm. The cessation of growth was indicated by a constancy in the readings. When glucose was present in the medium, the manometric reading was less than when it was absent. When proper precautions were taken, the calculated manometric reading, based on the real loss, approximated closely the corrected observed readings.

Oxygen pressures of varying amounts were used, and the effects noted. *Tr. lewisi* was more sensitive to increasing partial pressures of  $\text{O}_2$  than *L. tropica*. The germs were not killed by high tensions of  $\text{O}_2$  but their growth was inhibited, since subsequent incubation in air gave rich cultures.

A concentration of 50%  $\text{O}_2$  was the critical point for the growth of *L. tropica*. It was able to consume all of this  $\text{O}_2$  in the tubes used,

and returned nearly 34% of  $\text{CO}_2$ . At 60% of  $\text{O}_2$  concentration, the growth was inhibited, as shown by the lower manometric readings and by the lessened  $\text{O}_2$  consumption and  $\text{CO}_2$  production.

The parasites were found to be obligative aerobes. They required free  $\text{O}_2$  for growth and multiplication. Atmospheres of  $\text{N}_2$  or  $\text{H}_2$ , in which  $\text{O}_2$  was absent, inhibited growth. These elements, however, were not toxic.  $\text{CO}_2$  in concentrations greater than 20% was toxic for *Tr. lewisi*, even when an excess of  $\text{O}_2$  was present. *L. tropica* was less sensitive to  $\text{CO}_2$ , and partial pressures greater than 30% were necessary to produce injurious effects.

$\text{CO}_2$  was not essential to the life of these organisms. In the presence of alkali, growth was obtained, but the cultures, instead of forming a more or less uniform mass over the surface of the medium, showed only small isolated colonies. Desiccation of the surface appeared to be the cause of such poor growth.

The presence of defibrinated blood or serum was essential for the growth of the germs. No difference in the gas relations was noted by the substitution of serum for the blood.

The value of the respiratory quotient increased with the duration of the experiment, and this was interpreted as being due to secondary changes in the medium. The true respiratory quotient is obtainable when the culture is young and before such secondary changes can affect the result.

With blood agar medium, the corrected real respiratory quotient for *L. tropica* was found on the 7th day to be 0.839, and on the 28th day 0.907. Similarly, *Tr. lewisi* gave 0.735 on the 7th day and 0.888 on the 28th day. The average of 4 determinations (7-28 days) for *L. tropica* was 0.875, while for *Tr. lewisi* it was 0.822.

When glucose was present in the medium, the corrected real respiratory quotient was found to average 0.951 for *L. tropica* and 0.938 for *Tr. lewisi*.

The amount of  $\text{CO}_2$  taken up by the medium was considerably less when glucose was present, indicating a protein-sparing action.

The large amount of  $\text{CO}_2$  taken up by the plain blood agar medium indicated the formation of basic products either by deaminization or by decarboxylation, or both.

Per tube of culture, in 28 days, *Tr. lewisi* consumed all of the  $\text{O}_2$  in 277 c.c. of air, under ordinary conditions, i. e. 30 C. and 750 mm.; *L. tropica*, in the same time, utilized 212 c.c. of air. The latter on glucose blood agar, in 21 days, consumed the  $\text{O}_2$  in 354 c.c. of air.







